

Reply to the Comment on "Interfacial Lateral Electrical Conductance on Lipid Monolayers: Dose-Dependent Converse Effect of Alcohols"

Tadayoshi Yoshida^{*,†} and Issaku Ueda^{*,‡}

Department of Applied Chemistry, Nagoya Institute of Technology, Showaku, Nagoya 466-8555, Japan, and Anesthesia 112A, DVA Medical Center, and Department of Anesthesia, University of Utah, Salt Lake City, Utah 84148

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The Meniscus Problem. The formation of menisci has been a major problem in estimating the surface conductance of lipid monolayers. There are two places where menisci are formed: the electrode and the trough. For the electrodes, we eliminated the problem by submerging the horizontal electrode under the water surface.¹ This approach improved the surface-to-bulk conductance ratio, R_c , by about 20 ~ 100-fold, compared to the conventional parallel electrodes. In addition, the horizontal electrode suppressed the bulk conductance (base current) significantly.

The meniscus problem has been dealt with previously by Cavalli and Oliveira² and Shapovalov and Il'ichev.³ To prevent the formation of meniscus at the electrodes, Cavalli and Oliveira² placed the top of the parallel electrodes to the water surface. At the time of the monolayer-forming solution is added to the water, the surface oscillates and creates minute surface waves. The lipid molecules adhere to the top ends of the electrodes, which decreases the lateral conductance. Shapovalov and Il'ichev³ submerged the ring electrodes 1 mm below the water surface and reported that significant changes in ΔG were not observed at the formation of the monolayer. Their electrodes are constructed from platinum wire, therefore the surface area is small, and the pair were placed more than 10 mm apart. The R_c of their electrodes appears to be about 1/100 of ours.

The meniscus at the trough changes its size when the monolayer is formed. Then, the level of the water surface changes. We made the edge of the trough into a sharp angle and set the water surface at the edge.¹ This procedure practically eliminated the formation of the meniscus. The possibility of the shift of the level of the water surface after the formation of monolayer was measured by a Laser Displacement Meter (KEYENCE, LC-2420, Osaka, Japan). The change in the

position of the water surface after the formation of monolayer was within $\pm 3.0 \mu\text{m}$ and did not vary by the area of the trough (25, 100, and 150 cm^2). The change of 3.0 μm is negligible when compared to the distance between the water surface and the electrodes, 1 mm.

Cavalli and Oliveira² used a large surface-area trough to minimize the change of the height of the water surface when the monolayer is formed. However, because the top edges of the electrode touch the water surface, small change in the water surface position may distort the arrangement of the lipid molecules. Together with the low R_c values, these problems compromise the accuracy of the results.

The CO₂ Problem. When the water phase in equilibrium with room air is slowly mixed with a magnetic stirrer, the conductance measured by the horizontal electrode keeps decreasing over 20 min. When the mixing is stopped, the conductance slowly returned to the original value. These changes do not occur when the N₂ saturated water is used and the conductance is measured in the pure N₂ atmosphere. When the monolayer-forming liquid is added on the water surface, the spreading induces local mixing of water and affects the CO₂ concentration. The use of CO₂-free water and measurement under N₂ atmosphere is indispensable to obtain reliable and reproducible results.

The Alcohol Chain-Length Problem. The last comment assumes that partition of alcohols into the monolayer increases with the elongation of the hydrocarbon tail. Therefore, longer alcohols should increase the conductance more than shorter ones. The basic mechanism for the increase of the conductance depends on the hydrogen-bond breaking activity of alcohols to increase the probability of the collision between the next-neighbor phosphate moieties. This action depends on the membrane fluidity. The alcohol-lipid interaction force increases with the elongation of the alcohols hydrocarbon length. Then, the elongation of the hydrocarbon tail induces two opposing effects.

1. It increases membrane partitioning and increases fluidity.
2. The increased interaction between the lipid hydrocarbon and the alcohol hydrocarbon tails decreases fluidity.

The two factors work oppositely. We assume that the first effect is dominant with ethanol and propanol, and the second effect starts to take effect at butanol.

References and Notes

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- (2) Cavalli, A.; Oliveira, O. N., Jr. *Rev. Sci. Instrum.* **1995**, *66*, 5567.
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* To whom correspondence should be addressed.

[†] Department of Applied Chemistry, Nagoya Institute of Technology

[‡] Anesthesia 112A, DVA Medical Center, and Department of Anesthesia, University of Utah