

Determining the Concentration of Fatty Acid in an Aqueous Solution

Synopsis

Measuring the concentration of an aqueous fatty acid solution is easy with ADIFAB2— simply add an aliquot of the solution of interest to a cuvette containing ADIFAB2 and buffer and measure the fluorescence intensities at 550 and 457 nm (upon excitation at 375 nm).

Procedure

For details on measuring the ADIFAB2 ratio and calculating [FFA] and [ADIFAB2_{bound}] see [Determining the ADIFAB2 Ratio](#). Begin by measuring the value of the ADIFAB2 ratio without FA present (R_0) in a cuvette containing measuring buffer (20 mM HEPES, 140 mM NaCl, 5 mM KCl, 1 mM Na₂HPO₄, at pH 7.4). Add an aliquot of the fatty acid solution to the cuvette and measure R. The total fatty acid concentration in the cuvette is then [FFA] plus [ADIFAB2_{bound}] corrected for the fatty acid bound to the walls (Table 1). The concentration of the original fatty acid solution is the cuvette concentration multiplied by the dilution factor.

Table 1. Degree of FA binding to glass cuvettes.

Note: To quantify fatty acid binding to cuvettes of other materials see [Determining FA Wall Binding](#)

Fatty Acid	% Wall Binding			
	10	30	37	50
Laurate (12:0)			1.5	
Myristate (14:0)			8.5	
Palmitate (16:0)	7	20	22.3	30
Palmitoleate (16:1) 9 cis			8.1	
Stearate (18:0)			>50	
Oleate (18:1) 9 cis	18	20	21	29
Elaidate (18:1) 9 trans			21	
Petroselinate (18:1) 6 cis			21	
Vaccenate (18:1) 11 cis			21	
Linoleate (18:2) 9,12 cis	15	15	15	15
Linolenate (18:3) 9, 12, 15 cis	3	2	2	3
Arachidonate (20:4) 5, 8, 11, 14 cis	14	14	14	14

Example

ADIFAB2 was added to a cuvette containing 1.5 ml buffer (20 mM HEPES, 140 mM NaCl, 5 mM KCl, 1 mM Na₂HPO₄, at pH 7.4 and 22°C), so that the final ADIFAB2 concentration was 0.5 μM. The R_0 value was measured and found to be 0.085. Next, 0.005 ml of an oleate stock solution was added to the cuvette. The R value was measured and found to be 0.300. Using the equations and constants found in [Determining the ADIFAB2 Ratio](#), [FFA] = 74 nM and [ADIFAB2_{bound}] = 350 nM. Under these conditions about 19% of the sodium oleate was bound to the cuvette walls (Table

1) so the total amount of FA added to the cuvette was $(74 + 350)/0.81 = 523$ nM. Thus the stock concentration was $523 \text{ nM} \cdot 1.5 \text{ ml}/0.005 \text{ ml} = 157 \text{ } \mu\text{M}$.