# Development of an Equilibrium Dialysis Technique for Quantifying Starch-Lipid Complexes[1]

D. E. HAHN[2,3] and L. F. HOOD[2,4]

Cereal Chem. 64(2):77–80

**ABSTRACT**

A sensitive equilibrium dialysis technique for evaluating starch-lipid complexes was developed. The use of $^{14}C$-labeled lipid permitted accurate quantification using lipid concentrations that were soluble in water. An evaluation of membrane materials differing in chemical composition showed conventional regenerated cellulose dialysis film to be most effective in retaining dispersed starch while minimizing lipid adsorption to the membrane. A 12-hr dialysis was necessary to attain equilibrium. The complex was soluble in water and complex formation was reversible. The amount of bound lipid was affected by starch concentration.

The interaction between starch and lipid has been investigated by a variety of techniques including the Brabender viscoamylograph (Melvin 1979, Ohashi et al 1980), differential scanning calorimetry (Kugimiya et al 1980), decreases in starch solubility (Mercier et al 1980, Gray and Schoch 1962), and reduction in iodine-binding affinity (Ghiasi et al 1982). These methods are affected by differences in the extent of gelatinization (Van Lonkhuysen and Blankestijn 1974).

A more sensitive method for measuring binding in an aqueous system would be valuable for research investigating the physicochemical aspects of the starch-lipid complex. Equilibrium dialysis has been found to be sensitive in measuring fatty acid binding to albumin (Ashbrook et al 1972). This suggests that it might be a suitable technique for evaluating starch-lipid complexing.

The objectives of this study were to develop an equilibrium dialysis system for use with nongranular starch and lipid, to evaluate various membrane systems for compatibility with starch and lipid in an aqueous system, and to optimize experimental conditions for subsequent binding studies.

## MATERIALS AND METHODS

### Dialysis

A five-cell equilibrium dialyzer (Spectrum Medical Industries, Los Angeles, CA) was used. The dialysis cells consisted of two Teflon cell halves, each having a cell volume of 1.0 ml, separated by a semipermeable membrane. Five dialysis cells were stacked in a cell carrier with each cell separated from the others by a spring-loaded temperature adapter. The entire assembly was held together by three knurled nuts. The unit was modified by machining additional threads on each guide rod in order to apply sufficient pressure to insure cells free of leaks.

$^{14}C$ Palmitic acid (1.0 ml, 0.5 $\mu M$, 0.005 $\mu$Ci/ml; New England Nuclear, Boston, MA), in 0.1$M$ pH 7 citrate-phosphate buffer (Gomori 1955) + 0.02% $NaN_3$ was added to each cell using a syringe equipped with a blunt-tipped needle. This was equilibrated against 1.0 ml of 0.1$M$ pH 7 citrate-phosphate buffer + 0.02% $NaN_3$. The cells were placed in a 20° C water bath and rotated at 20 rpm, with 25-$\mu$l duplicate aliquots removed from each cell half at seven intervals ranging from 0.5 to 12 hr. Aliquots were added to 10 ml of scintillation fluid (Liquiscint, National Diagnostics, Somerville, NJ) and were analyzed using a model 3385 liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, IL).

### Membrane Evaluation

Membranes differing in chemical composition and porosity were evaluated for their starch-retaining ability by adding 1.0 ml of 0.1% unmodified dent corn starch that had been autoclaved for 30 min at 20 psi to one cell half and adding 1.0 ml of distilled deionized water to the opposite cell half. Spectra Por 2 and 6 regenerated cellulose membranes (Spectrum Medical Industries, Los Angeles, CA) were cut into 40 × 80 mm segments and soaked in distilled water for 15 min and 30% methanol for 20 min. Other membranes used to separate cell halves were supplied as 45-mm disks and were treated by soaking in alcoholic solvents as indicated in Table I. All membranes were given a final water rinse before the dialysis experiment.

Starch permeability of the membranes was determined by withdrawing aliquots from the cell half opposite the original starch addition after the cells had rotated for 12 hr. The phenol-sulfuric acid procedure (DuBois et al 1956) was used to quantitate the amount of starch passing through the membrane.

Lipid permeability of the membranes was evaluated by adding 1.0 ml of $^{14}C$ palmitic acid (59 $\mu$Ci/$\mu$mole, 0.005 $\mu$Ci/ml) to one cell half and dialyzing against 1.0 ml of distilled deionized water for 12 hr at 20 rpm. Aliquots (0.4 ml) were withdrawn from both cell halves and analyzed by liquid scintillation. After the dialysis cell was drained of remaining solution, it was opened and the membrane was blotted dry. The membrane was placed in a scintillation vial containing 10 ml of scintillation fluid, vortexed, and counted with the liquid scintillation counter.

The amount of lipid adsorbed to the membrane during dialysis was evaluated. Fatty acids (lauric, palmitic, and stearic) were added either to the cell half with added starch or the opposite cell half. In all cases, the initial fatty acid concentration was 0.5 $\mu M$. The starch concentration was 0.05%. All solutions contained 0.1$M$ pH 3 citrate-phosphate buffer + 0.02% $NaN_3$. Equilibration was carried out at 60° C. After 12 hr, the contents were removed from the cells and the membranes were blotted dry. Lipid adsorbed to the membrane was determined by placing the membrane in a vial containing 10 ml of scintillation fluid and measuring radioactivity with the scintillation counter.

### Starch Concentration

The effect of starch concentration on the extent of complex formation was determined. Aqueous corn starch dispersions of 0.01, 0.02, 0.1, and 0.2% were autoclaved at 20 psig for 30 min. Aliquots (0.5 ml) of each solution were added to one cell half of a dialysis cell fitted with a Spectra Por 2 membrane along with 0.5 ml of 1.0 $\mu M$ stearic, palmitic, or lauric acid in 0.2$M$ pH 7 citrate-phosphate buffer + 0.04% $NaN_3$. This resulted in the solution in this cell half containing 0.5 $\mu M$ fatty acid, 0.1$M$ buffer, 0.02% $NaN_3$, and a starch content of 0.005–0.1%. This mixture was equilibrated against 1.0 ml of 0.1$M$ pH 7 citrate-phosphate buffer

[1]Taken in part from a thesis submitted by D. E. Hahn in partial fulfillment of the requirements for the Ph.D. degree.

[2]Department and Institute of Food Science, Cornell University, Ithaca, NY 14853.
[3]Present address: Sandoz Nutrition Corporation, Minneapolis, MN 55416.
[4]Present address: The Pennsylvania State University, University Park, PA 16802.

This manuscript was prepared for electronic processing.

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. American Association of Cereal Chemists, Inc., 1987.

+ 0.02% $NaN_3$ at 40° C for 12 hr. Duplicate 0.4-ml aliquots were removed from each cell half and counted with the liquid scintillation spectrometer. All experiments were conducted in duplicate cells.

### Dissociation

Studies were conducted to determine if starch-lipid complex formation was reversible in dilute aqueous systems. A starch-myristic acid complex was allowed to form within eight identically prepared dialysis cells by adding 0.5 ml of 0.01% dispersed corn starch in water and 0.5 ml of 1 $\mu M$ $^{14}C$ myristic acid in 0.2$M$ pH 7 citrate-phosphate buffer + 0.04% $NaN_3$ to one ("starch") cell half, and adding 1.0 ml of 0.1$M$ pH 7 citrate-phosphate buffer + 0.02% $NaN_3$ to the opposite ("buffer") cell half. After 12 hr of incubation at 40° C, two 0.4-ml aliquots were removed from the buffer cell half and were counted with the liquid scintillation spectrometer. The residual liquid in the buffer cell half was removed, and 1.0 ml of fresh 0.1$M$ pH 7 citrate-phosphate buffer + 0.02% $NaN_3$. was added. This procedure was repeated seven times in the following 150 hr, at which time aliquots removed from the buffer cell half contained less than 80 counts per minute/0.4 ml.

## RESULTS AND DISCUSSION

### Membrane Evaluation

Membranes differing in composition and pore size were evaluated for their starch and lipid permeability and lipid adsorption capabilities. The ideal membrane for use with this

**TABLE I**
**Starch and Lipid Permeability and Lipid Adsorption of Selected Dialysis Membranes**

| Membrane | Pore Size ($\mu$m) | Pretreatment[a] | Permeable Starch[b] (%) | Permeable Lipid[c] (%) | Membrane-Adsorbed Lipid[d] (%) |
|---|---|---|---|---|---|
| Teflon (Polytetrafluroethylene) | 0.5[e] | none | 0.1 | 11 | 19 |
| | | 100% MeOH[l] | 6.0 | ··· | ··· |
| | 0.45[f] | none | 2.9 | 42 | 32 |
| | | 100% MeOH[l] | 4.6 | 38 | 67 |
| Teflon with polypropylene backing | 1.0[g] | none | 2.5 | 38 | 42 |
| | | 100% MeOH[l] | 5.1 | 41 | 80 |
| | 1.0[h] | none | 1.0 | 27 | 60 |
| | | 100% MeOH[l] | 4.1 | 27 | 82 |
| | 0.5[g] | none | 0 | 44 | 34 |
| | | 100% MeOH[l] | 15.8 | 50 | 75 |
| | 0.2[g] | none | 0 | 17 | 19 |
| | | 100% MeOH[l] | 12.5 | 36 | 80 |
| Polyvinylidene fluoride | | | | | |
| Hydrophobic | 0.45[e] | none | 0.3 | 9 | 57 |
| | | 100% MeOH[l] | 22.9 | ··· | ··· |
| | 0.22[e] | none | 2.4 | 8 | 69 |
| | | 100% MeOH[l] | 18.6 | ··· | ··· |
| Hydrophilic | 0.45[e] | none | 12.5 | ··· | ··· |
| | 0.22[e] | none | 14.1 | ··· | ··· |
| Polyvinylchloride | 0.5[e] | none | 24.4 | ··· | ··· |
| | 0.45[i] | none | 39.8 | ··· | ··· |
| Cellulose acetate | | | | | |
| Hydrophilic | 1.2[h] | none | 47.5 | 49 | 59 |
| | 0.45[h] | none | 42.9 | ··· | ··· |
| | 0.2[h] | none | 16.8 | 37 | 53 |
| | 0.01[h] | none | 0.3 | 50 | 71 |
| Hydrophobic | 0.45[i] | none | 39.6 | 52 | 68 |
| | 0.45[f] | none | 0 | 23 | 81 |
| | | 15% EtOH[m] | 11.1 | 41 | 71 |
| | 0.2[f] | none | ··· | 49 | 95 |
| | | 15% EtOH[m] | ··· | 52 | 91 |
| Nitrocellulose | 12.0[h] | none | 26.9 | 46 | ··· |
| Polyamide | 0.2[f] | none | 19.9 | ··· | ··· |
| Polyester | 0.2[j] | none | 12.8 | 44 | 23 |
| Polycarbonate | 12.0[j] | none | ··· | 50 | 6 |
| | 0.4[j] | none | 25.4 | 45 | 24 |
| High-temperature aromatic polymer | 0.45[i] | none | 38.1 | 41 | 57 |
| Regenerated cellulose | 1.0[h] | none | 40.4 | 48 | 28 |
| | 0.45[h] | none | 12.8 | ··· | ··· |
| | 0.2[h] | none | 14.0 | 47 | 23 |
| | 0.01[h] | none | 0.3 | 50 | 37 |
| 50,000 molecular weight cut-off[k] | ··· | 30% EtOH[l] | 0.2 | 49 | 9.5 |
| 12,000 molecular weight cut-off[k] | ··· | 30% EtOH[l] | 0.3 | 49 | 7.5 |

[a] MeOH = methanol; EtOH = ethanol.
[b] Mean percent of total carbohydrate recovered in palmitic acid cell half after 12 hr, $n = 2$.
[c] Mean percent of nonadsorbed palmitic acid recovered in carbohydrate cell half after 12 hr, $n = 2$.
[d] Mean percent of total palmitic acid, $n = 2$.
[e] Millipore Corporation, Bedford, MA.
[f] Sartorious Filter Inc., Hayward, CA.
[g] Micro Filtration Systems, Dublin, CA.
[h] Schleicher and Schuell, Keene, NH.
[i] Gelman Sciences, Inc., Ann Arbor, MI.
[j] Nuclepore Corp., Pleasanton, CA.
[k] Spectrum Medical Industries, Los Angeles, CA. Molecular weight cutoffs 50,000 and 12,000 are for Spectra Por 6 and 2, respectively.
[l] 20° C.
[m] 50° C.

system would retain all of the dispersed starch, permit the rapid diffusion of lipid, adsorb no lipid, and act as a gasketing material, reducing the possibility of fluid leakage.

The results from all membranes tested are summarized in Table I. Pretreatment of the hydrophobic membranes with methanol or ethanol resulted in greater starch permeability and, in most cases, higher amounts of lipid adsorbed to the membrane. After evaluating all parameters, Spectra Por 2 and 6 were judged to be the most acceptable. Both yielded at least 98% recovery of the added lipid. Spectra Por 2 was selected for further study because it was thinner than conventional regenerated cellulose membranes. Dialysis rates are inversely proportional to the square of membrane thickness, other factors being equal (Daniel 1950).

The time necessary to equilibrate an aqueous solution of palmitic acid using Spectra Por 2 was about 12 hr (Fig. 1). The initial rate of dialysis was high when the concentration differential was greatest. The use of higher temperatures or lower molecular weight lipids resulted in shorter equilibration times, but a constant 20° C for a 12-hr incubation time was adopted for all subsequent dialysis experiments.

**Method Evaluation**

Calculated binding values rely on the difference in the number of counts present in solution on opposite sides of the membrane. If precipitation occurred upon complex formation, a decline in the soluble starch concentration would be apparent. After incubating starch and lipid at 20° C for 12 hr, the solution remained clear, developing no precipitate or flocculate even though complexing had occurred. Other investigators have stated that the formation of a starch-lipid complex results in precipitation, and the quantity of precipitated starch (or the reduction in "soluble" starch concentration) has been used as an index of the extent of complex formation (Strandine et al 1951, Hoover and Hadziyev 1981). Apparently, the concentration of starch was sufficiently low in our experiments to minimize precipitation.

The effect of adding fatty acid, to the same or opposite side as the starch, on membrane-bound lipid is shown in Figure 2. The amount of lipid found on the membrane was greater when fatty acid was added to the side opposite the carbohydrate solution. It is conceivable that because the initial point of contact between starch and lipid is the membrane surface, some of the complex that is formed could be trapped within the membrane. The smaller amount of lipid entrapped on the membrane when it was added to the same side as the starch permitted the concentration of fatty acid to remain closer to that originally intended.

The amount of membrane-bound lipid was influenced by the fatty acid molecular weight. With the opposite side addition, the amount of membrane-bound lipid increased as the molecular weight of the fatty acid increased. Apparently, more stearic acid attempted to pass through the membrane than lauric acid, as stearic acid has a greater affinity for starch. Because a greater quantity of stearic acid passed through the membrane, there was a greater possibility for more stearic acid to be entrapped within the membrane.

When starch and lipid were added to the same side of the cell, the greater affinity of stearic acid for starch caused less of this lipid to interact with the membrane. Consequently, lipids which bound less strongly to starch were found adsorbed to the membrane to a greater extent. The problem of membrane-bound lipid was minimized by adding starch and lipid to the same side of the

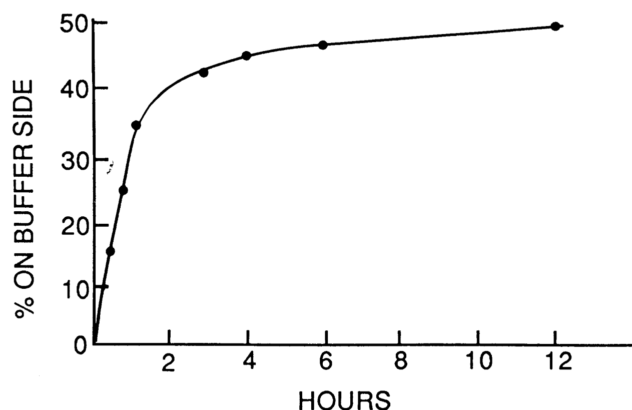


**Fig. 1.** Equilibration of 0.5 $\mu M$ palmitic acid at 20° C, pH 7, 20 rpm, using a Spectra Por 2 membrane.

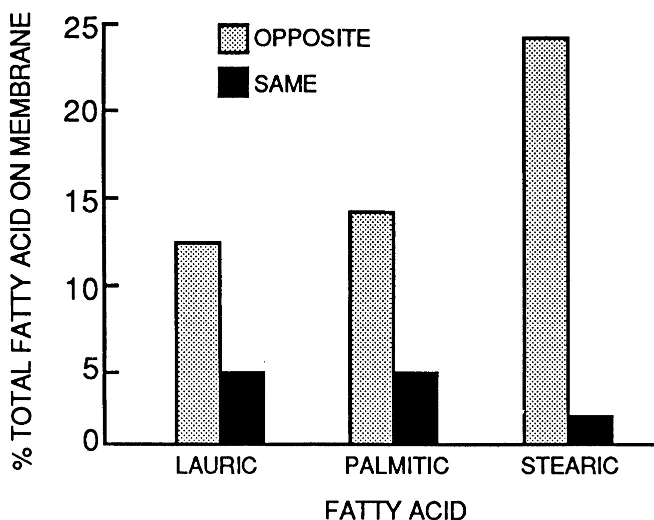


**Fig. 2.** Effect of adding fatty acids to the cell half containing starch (same side addition) or the opposite cell half containing no starch on the quantity of membrane-bound lipid.

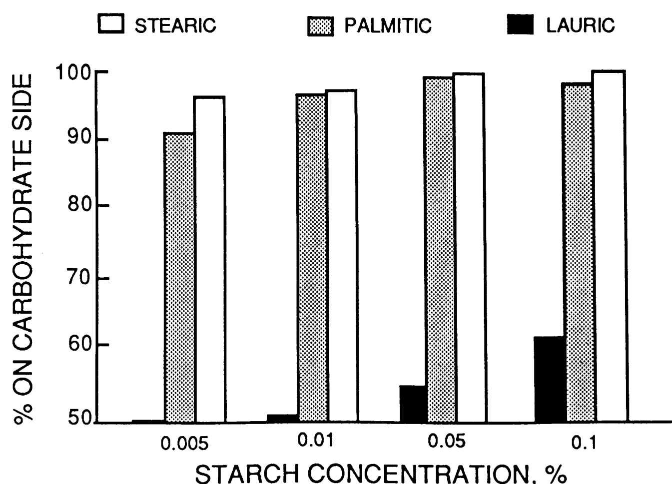


**Fig. 3.** Effect of starch concentration on fatty acid complexing. Starch and fatty acid added to the same cell half at the onset of dialysis.

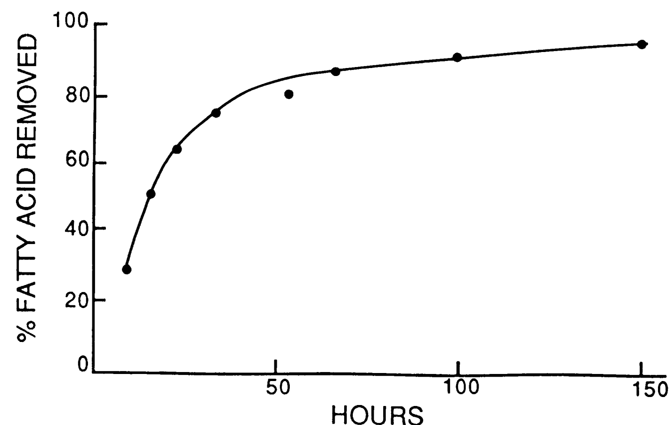


**Fig. 4.** Dissociation of the starch-lipid complex at 40° C, pH 7, with 0.5 $\mu M$ myristic acid.

membrane at the onset of dialysis.

The influence of starch concentration on the amount of fatty acid bound after same side addition is shown in Figure 3. A relatively high (0.1%) starch level was necessary to ensure that at least 60% of the lauric acid was present on the carbohydrate side after equilibration. However, at this concentration, essentially all of the palmitic and stearic acid added to the cell was bound to the starch. By reducing starch concentration to 0.005%, differences were shown between stearic and palmitic acid binding ability. However, at 0.005% starch, lauric acid complexing could not be detected.

Stearic acid had the highest binding affinity for starch. To determine how the complexing ability of this lipid is influenced by reaction conditions such as pH and temperature, it would be necessary to ensure that any effect was not masked by too high a starch concentration. At low starch concentrations (0.005%), the dialysis system was very sensitive to the effects of varying experimental conditions. The drawback to using such a low starch concentration is that lauric acid will show little or no effect.

Starch-lipid complex formation was a reversible reaction (Fig. 4). Repeated removal of buffer from the buffer cell half and replacement with fresh buffer resulted in essentially all of the myristic acid originally added to the carbohydrate side being removed from the buffer side. After 140 hr, only 6% of the counts originally added to the cell were not removed. The lipid removed from the buffer cell half was in the free or unbound state, as dispersed starch cannot pass through the dialysis membrane.

## CONCLUSION

An equilibrium dialysis technique was developed to measure fatty acid-starch complexing. Of all membranes evaluated, conventional regenerated cellulose was best, allowing lipid equilibration with low membrane adsorption. Membrane-adsorbed lipid levels were minimized by adding starch and lipid to the same cell half at the onset of dialysis. A 12-hr incubation period was used to assure lipid equilibration.

Under the conditions used in this study, the starch-lipid complex did not precipitate. Following equilibration at 40° C, it was possible to remove nearly all the lipid added to the cell by repeated removal of buffer from the buffer cell half and replacement with fresh buffer. This suggests that starch-lipid complexation is reversible at 40° C.

Starch concentration influenced the ability of lauric acid to bind starch. A low starch concentration should be used to detect differences in the binding potential of higher molecular weight fatty acids. This technique will be very useful in studying factors influencing starch-lipid complexing.

## LITERATURE CITED

ASHBROOK, J. D., SPECTOR, A. A., and FLETCHER, J. E. 1972. Medium chain fatty acid binding to human plasma albumin. J. Biol. Chem. 247:7038.

DANIEL, F. K. 1950. Dialysis and electrodialysis. Page 1 in: Encyclopedia of Chemical Technology. Vol. 5. R. F. Kirk and D. F. Othmer, eds. Interscience Publishers: New York.

DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A., and SMITH, F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350.

GHIASI, K., VARRIANO-MARSTON, E., and HOSENEY, R. C. 1982. Gelatinization of wheat starch. II. Starch-surfactant interaction. Cereal Chem. 59:86.

GOMORI, G. 1955. Preparation of buffers for use in enzyme studies. Methods Enzymol. 1:138.

GRAY, V. M., and SCHOCH, T. J. 1962. Effects of surfactants and fatty adjuncts on the swelling and solubilization of granular starches. Staerke 14:239.

HOOVER, R., and HADZIYEV, D. 1981. Characterization of potato starch and its monoglyceride complexes. Staerke 33:290.

KUGIMIYA, M., DONOVAN, J. W., and WONG, R. Y. 1980. Phase transitions of amylose-lipid complexes in starches: A calorimetric study. Staerke 32:265.

MELVIN, M. A. 1979. The effect of extractable lipid on the viscosity characteristics of corn and wheat starches. J. Sci. Food Agric. 30:731.

MERCIER, C., CHARBONNIERE, R., GREBAUT, J., and GUERIVIERE, J. F. 1980. Formation of amylose-lipid complexes by twin-screw extrusion cooking of manioc starch. Cereal Chem. 57:4.

OHASHI, K., GOSHIMA, G., KUSUDA, H., and TSUGE, H. 1980. Effect of embraced lipid on the gelatinization of rice starch. Staerke 32:54.

STRANDINE, E. J., CARLIN, G. T., WERNER, G. A., and HOPPER, R. P. 1951. Effect of monoglycerides on starch, flour and bread: A microscopic and chemical study. Cereal Chem: 28:449.

VAN LONKHUYSEN, H., and BLANKESTIJN, J. 1974. Interaction of monoglycerides with starches. Staerke 26:337.

[Received December 12, 1985. Accepted October 2, 1986.]