method for evaluating hemolysis liabilities of sterilized plastics. From the results of these tests, the polypropylene devices would have to be considered the best candidates for making a medical product which would undergo ethylene oxide sterilization. However, techniques must be evaluated to prove the effectiveness of longer degassing times, higher temperatures, and the effects of vacuum. It would appear from these results that further testing is necessary to establish the actual percentage of hemolysis that occurs for a particular concentration of ethylene oxide. In any event, it is sufficient to say that hemolysis liabilities do exist from the sterilization of medical plastics of many different types.

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*Keyphrases*

Ethylene oxide sterilization
Sterilization of plastic devices
Plastics—entrapment
Solubility in phthalic esters
Glass—entrapment
Toxicity of ethylene oxide
Tissue culture
Hemolysis—induced

# Surface Films of Soybean Lecithin II

## Interactions Between Lecithin and Lipid Substances in Mixed Monomolecular Films

By G. TOROSIAN* and A. P. LEMBERGER

**Mixed films of lecithin with the fatty acids (stearic, elaidic, and oleic) as well as with the glycerol esters of mono-, di-, and triglyceride have been investigated. Evaluation of these systems has been made utilizing two properties of surface films, their mean molecular areas and their collapse points. It would appear from this investigation that in mixed film systems, lecithin–stearic acid and lecithin–monoglyceride are miscible, but without a significant degree of interaction and as such, form ideal two-dimensional liquids. Lecithin–oleic and lecithin–elaidic acids have been shown to be miscible in mixed film systems but with a significant degree of interaction so that they may be considered to be nonideal two-dimensional liquids. The data for the lecithin–di- and triglyceride systems did not conclusively show whether these systems were ideal or nonideal two-dimensional liquids in mixed surface films.**

THE IMPORTANCE of phospholipids in biological membranes has been discussed by Bangham (1). Among the phospholipids, lecithin is considered particularly important since it aggregates into sheet-like micelles which are capable of further accommodating lipids, phospholipids, and sterols (2, 3). The mixed film system of lecithin–cholesterol, first investigated by Dervichian (4), showed the ability of cholesterol to condense lecithin to a smaller cross-sectional area than it exhibits alone. Van Deenen (5) indicated the molecular proportions of cholesterol to phosphotides of animal red cell membranes

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to be 1:1, thus increasing the biological significance of Dervichian's lecithin–cholesterol mixed film observations. Van Deenen (5) verified the ability of cholesterol to condense lecithin using synthetic lecithin containing saturated fatty acid moieties alone and lecithin with unsaturated fatty acid moieties. The condensing effect of cholesterol was more pronounced when in a mixed film in conjunction with the synthetic lecithin containing unsaturated acyl groups.

Lipid materials with which lecithin may be associated in nature include fatty acids and glyceryl esters. Dervichian (6) and Desnuelle (7) have observed interactions between phospholipids and other lipid substances. Yeadon *et al.* (8) studied the emulsification properties of lecithin. They were able to show that purified lecithin alone was an inefficient emulsifier while purified lecithin in conjuction with other lipids in some cases led to improved emulsions. The implications of mixed film interactions between lecithin and these materials, both in nature and in pharmaceutical systems such as emulsions, are of broad interest. Hence this study was undertaken to examine carefully the nature and degree of lecithin interactions with several fatty materials.

## EXPERIMENTAL

**Materials**—Lecithin used in this study was prepared from soybean concentrate[1] and purified after the method of Saunders and Perrin (9, 10). Stearic and oleic acids, obtained from Mann Research Laboratories, were chromatographically pure and were used without further treatment. The elaidic acid used in this study was obtained from K and K Laboratories and was further purified by recrystallization twice from ethanol–water mixture. The pure elaidic acid had a melting point range from 44–45°. The saturated (primarily stearoyl with some palmitoyl moieties) mono-, di-, and triglycerides were obtained from K and K Laboratories as a mixture and were purified and separated after the chromatographic method of Higuchi *et al.* (11).

**Apparatus and Experimental Procedure**—The surface balance employed was that developed by Poulsen and Lemberger (12). Concentrated solutions of the pure components for a particular mixed film series were prepared separately using dried redistilled reagent benzene as the solvent. Specific volumes of these stock solutions were combined, maintaining the final volume constant, so that the mole fraction of the second component, any compound mixed with lecithin, increased for each mixed film determination within a particular system. These solutions containing the specified mole fraction of each component were then layered on the surface of double-distilled water contained in the trough of the film balance by using a Hamilton syringe with a Chaney adapter. The volume of the injection was held constant throughout this study at 0.1 ml. Temperature was held constant throughout a particular compression and all runs were made at 25 ± 0.5°.

Layering of the material was performed slowly to avoid dissolution of the wax coating on the trough by the benzene. The resulting mixed film was compressed manually until the film collapsed, with readings taken at specific trough areas. This allowed the construction of the $\pi$–$A$ isotherms, where $\pi$ equals the film pressure and $A$ equals the area occupied by the molecules. The time intervals between individual compressions were constant throughout the course of a single run, but did vary with different mixed films from 1–5 min., depending upon the components in the mixed films. Molecular cross-sectional areas for lecithin, obtained by extrapolation to $\pi = 0$, were reproducible within less than 2% for a given lot of lecithin. In general, the study of each system was completed with the same lot of lecithin. Molecular cross-sectional areas for mixed film systems were reproducible within 1–2 Å.$^2$. This allowed the construction of the $\pi$–$A$ isotherms, where $\pi$ = the film pressure, and $A$ = the area occupied by the molecules.

## RESULTS AND DISCUSSION

**Construction of $\pi$–A Isotherms**—Figures 1 and 2 are representative families of curves which graphically indicate what occurs as the mole fraction of lecithin is decreased while increasing that of the second component. While systems containing lecithin with stearic acid, oleic acid, elaidic acid, mono-, di-, and triglycerides were investigated, only representative isotherms for lecithin–stearic acid and lecithin–oleic acid are presented since they show what typically occurs in these systems.

In general, it can be seen that as the mole fraction of the second component increases, the curve for the mixed film shifts along the area/molecule axis. Reading the plots from right to left, each succeeding curve represents a further increase in the mole fraction of the second component, until on the extreme left is represented the pure second component. Films of pure natural lecithin exhibit an expanded liquid character, which when diluted

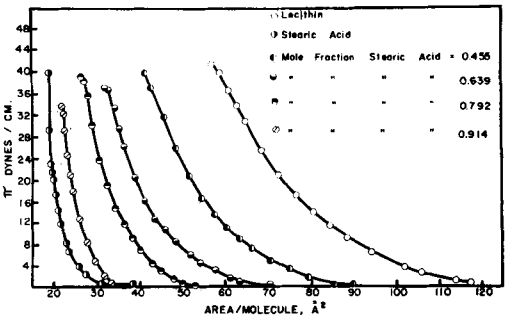


*Fig. 1—Representative compression isotherms for lecithin–stearic acid systems. Area/molecule axis based on total number of molecules.*

[1] Azolectin, Associated Concentrates, Inc., Woodside, N. Y.

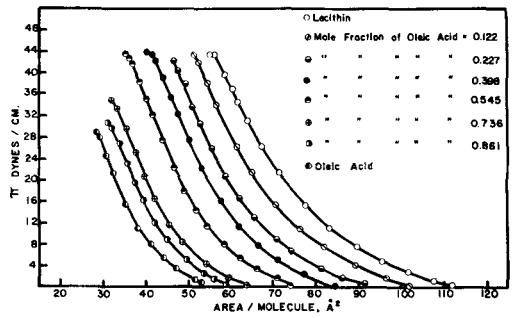


*Fig. 2—Represenative compression isotherms for lecithin–oleic acid system. Area/molecule axis based on total number of molcules.*

with a second component whose mono-molecular film exhibits a more condensed solid state, results in a mixed film which is a hybrid in its character with respect to the two pure components. The degree to which this hybridization occurs is dependent upon the mole fraction of the second component and on the specific characteristics of the second component. This is demonstrated in Fig. 1, which shows the lecithin–stearic acid system in which the gradual progression of the expanded liquid state of pure lecithin to the more condensed solid state of component 2, stearic acid, takes place. These rather general observations hold equally well for those other more condensed components when mixed with lecithin such as the mono-, di-, and triglycerides.

On the other hand, when 2 components are mixed, both of which when pure show an expanded liquid character, the resulting mixed film retains its expanded liquid characteristic from pure component 1 all the way to pure component 2. This can be seen in Fig. 2 for the lecithin–oleic acid system and is also true for lecithin–elaidic acid system.

In addition to the change in the shape of the curve and the progression of the curves toward smaller cross-sectional areas as the mole fraction of component 2 is increased, there is another property of these films which may change and that is the point at which the mixed film collapses. The collapse point is experimentally taken to be the highest film pressure attainable. In Fig. 1, the lecithin–stearic acid system shows little change of the collapse point, while Fig. 2 for lecithin–oleic acid system shows a gradual reduction in the collapse pressure from the high point exhibited by pure lecithin to the low collapse pressure indicated for pure oleic acid, particularly above the mole fraction of 0.55. This property is again observed in the systems containing elaidic acid, di-, and triglycerides. The pure monoglyceride, like pure stearic acid, has a collapse pressure as high as pure lecithin itself, and one observes little change in the collapse pressure for the mixed film systems of lecithin–stearic acid and lecithin–monoglyceride.

In an attempt to characterize the systems of lecithin with stearic acid, oleic acid, elaidic acid, mono-, di-, and triglycerides, plots of the extrapolated mean cross-sectional area *versus* mole fraction of the second component were constructed. The extrapolated mean cross-sectional areas were obtained by extrapolating the linear portions of the isotherms to $\pi = 0$ on the area/molecule axis. The extrapolated mean cross-sectional area obtained in this manner represents the average area occupied by the molecule in the particular system at zero pressure. The mean cross-sectional areas, on the other hand, were obtained by reading the area/molecule on the $x$-axis at the specified pressure. This results in a mean cross-sectional area for the molecule at the pressure specified and reflects the average area occupied by the molecules at that specific pressure for that specific system.

When these properties are plotted against the mole fraction of the second component, a linear relationship results if the cross-sectional areas are additive implying the existence of an ideal two-dimensional liquid. This is seen to be the case in Fig. 3 for the lecithin–stearic acid system. This relationship is shown to hold as well for the mean cross-sectional area at film pressures of 10–30 dynes/cm. as seen in Fig. 4. Figures 5 and 6 for the lecithin–monoglyceride system also seem to be linear and closely resemble the lecithin–stearic acid system. This is not, however, the case for the lecithin–oleic acid and the lecithin–elaidic acid systems. Figures 7 and 8 show that most of the points fall below the theoretical line. This is further demonstrated in Figs. 9 and 10 for the film pressures from 4 to 20 dynes/cm. This implies that the cross-sectional areas are less than additive for these systems.

**Apparent Linearity of Lecithin–Di- and Triglyceride**—The lecithin–di- and triglyceride

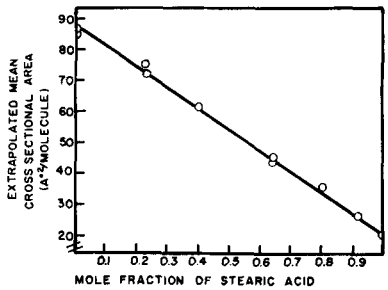


*Fig. 3—Extrapolated mean cross-sectional area* versus *mole fraction of component 2, stearic acid, of lecithin–stearic acid system. Key: ——, theoretical; ○, experimental.*

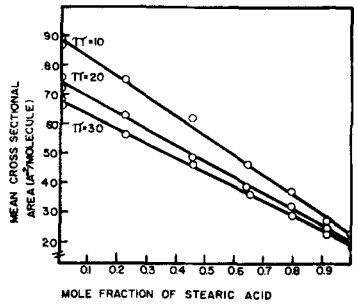


*Fig. 4—The mean cross-sectional areas taken at different π values* versus *mole fraction of stearic acid for lecithin–stearic acid system. Key: ——, theoretical; ○, experimental.*

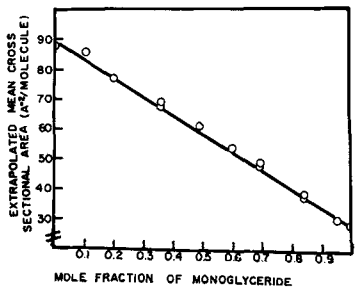


*Fig. 5—Extrapolated mean cross-sectional area* versus *mole fraction of monoglyceride for lecithin–monoglyceride system. Key: ——, theoretical; ○, experimental.*

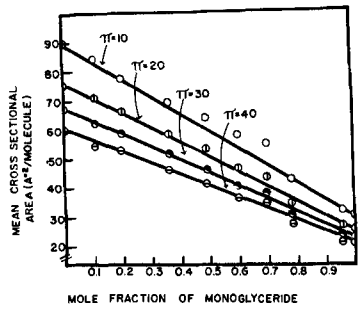


*Fig. 6—Mean cross-sectional area at different π values* versus *mole fraction of monoglyceride for lecithin–monoglyceride system. Key: ——, theoretical; ○, experimental.*

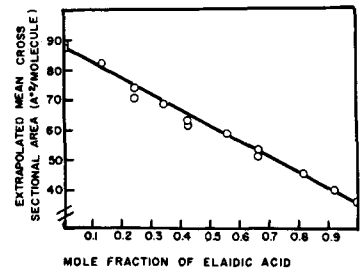


*Fig. 7—Extrapolated mean molecular cross-sectional areas* versus *mole fraction of elaidic acid for lecithin–elaidic acid system. Key: ——, theoretical; ○, experimental.*

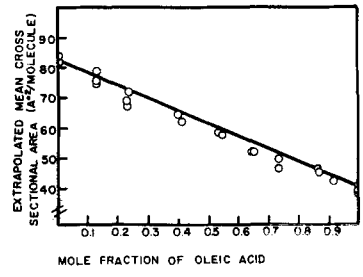


*Fig. 8—The extrapolated mean cross-sectional area* versus *the mole fraction of oleic acid for lecithin–oleic acid system. Key: ——, theoretical; ○, experimental.*

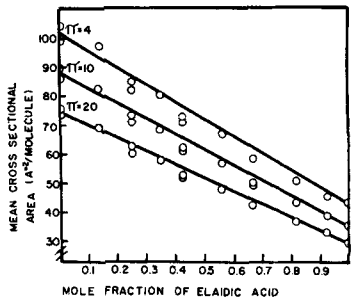


*Fig. 9—Mean cross-sectional areas at different π valves values* versus *mole fraction of elaidic acid for lecithin–elaidic acid system. Key: ——, theoretical; ○, experimental.*

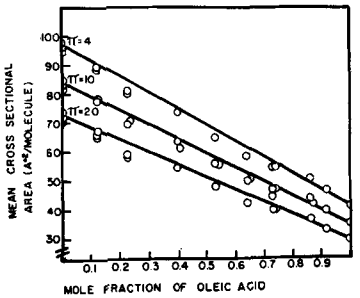


*Fig. 10—Mean cross-sectional area at different π values* versus *the mole fraction of oleic acid in the lecithin–oleic acid system. Key: ——, theoretical; ○, experimental.*

systems show a linear relationship between the extrapolated mean cross-sectional area and the mean cross-sectional area *versus* the mole fraction of the second component similar to that observed for the lecithin–monoglyceride system. This would seem to indicate that the mean molecular cross-sectional area for the lecithin–diglyceride and triglyceride mixed films are also additive. However, it will be shown that this evidence alone is insufficient to confirm this first impression.

According to Crisp (13), two components of a mixed film are immiscible if the components remain separate and distinct from each other, when they form as for example, surface micelles, then the following equation is obeyed:

$$A_T = A_1N_1 + A_2N_2 \qquad \text{(Eq. 1)}$$

where $A_T$ = total area, $A_1$ = area of component 1, $N_1$ = the mole faction of component 1, $A_2$ = area of component 2, and $N_2$ = the mole faction of component 2. Molecular interaction and miscibility result when Eq. 1 is not obeyed, yet agreement with Eq. 1 does not absolutely indicate immiscibility since components may be miscible with each other yet have little or no interaction between them, and Eq. 1 may still be observed for these ideal two-dimensional solutions. Most mixed film systems may have some range of miscibility and according to Crisp (13) one may determine the limiting solubility of component 2 in component 1, as well as the partial molar area of component 2, the value of which serves to give some indication as to the ex-

tent of the interaction. Crisp (13) defines the partial molar area as:

$$\bar{A} = -RT \frac{d \ln N'_s}{d\pi'} - RT \frac{d \ln f_s'}{d\pi'} \quad \text{(Eq. 2)}$$

where $\bar{A}$ = partial molar area, $R$ = gas constant, $T$ = absolute temperature, $N_s'$ = mole fraction of the second component being squeezed out of the films. $\pi'$ = film pressure at which the second component is being squeezed, and $f_s'$ = activity coefficient of the second component at the point of being squeezed out. If the activity coefficient is reasonably constant, one may plot $\ln N_s'$ *versus* $\pi'$. The partial molar area may be calculated from the slope of the line, and limiting solubility of component 2 in component 1 may be obtained from the point at which the linear portions at high $N$ values, as reported by Crisp, intersect.

**Evaluation of Partial Molar Areas**—Figures 11–14 are examples of plots of the film pressure at the collapse point *versus* log $N_2$. These plots, made after the method of Crisp (13), do not show an immiscible region at high $N_2$ values. There is, however, a linear segment in these plots corresponding to the region in which collapse film pressure increases as $N_2$ decreases up to a limiting value. The slope of the first portion allows the calculation of the partial molar area, $\bar{A}$, of component 2 in the system. $\bar{A}$ values for the various additives are listed in Table I along with their extrapolated cross-sectional areas and the actual molecular area at the collapse pressure of these components obtained when their films were cast alone. There is a reduction in the partial molar areas with respect to the extrapolated values, indicating that the interaction between lecithin and these second components does occur, and hence the mixed films appear to be condensed with respect to the areas of either component alone. Crisp describes this region as the region where component 2 is miscible with component 1, consequently the film pressure at the collapse point increases. In the systems reported here the mole fraction of component 2 was observed to reach a critical value at which the enhancement of collapse pressure was altered. At $N_2$ levels below the critical value in oleic and elaidic acid systems the collapse point became essentially constant and was essentially the same as for pure lecithin. In the lecithin–triglyceride system, the collapse points seemed to increase gradually with a reduction of $N_2$ until the collapse point of lecithin was reached while the diglyceride system demonstrated a maximum collapse pressure at the critical value. Since, according to Gaines (14) Eq. 2 holds only in the region where

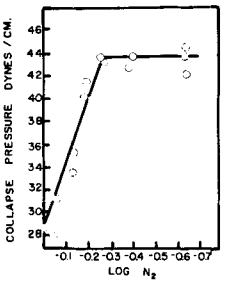


*Fig. 11—A plot of the logarithm of the mole fraction of oleic acid* versus *the film pressure at the collapse point for the lecithin–oleic acid mixed film system.*

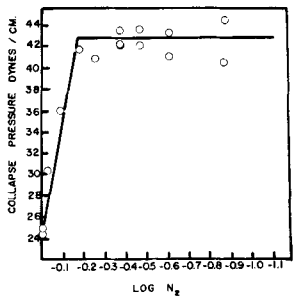


*Fig. 12—A plot of the logarithm of the mole fraction of elaidic acid* versus *the film pressure at the collapse point for the lecithin–elaidic acid mixed film system.*

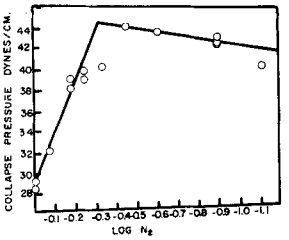


*Fig. 13—A plot of the logarithm of the mole fraction of diglyceride* versus *the film pressure at the collapse point for the lecithin–diglyceride mixed film system.*

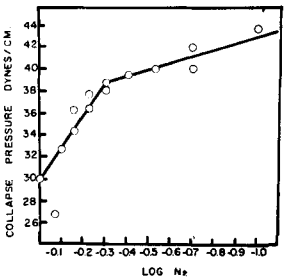


*Fig. 14—A plot of the logarithm of the mole fraction of triglyceride* versus *the film pressure at the collapse point for the lecithin–triglyceride mixed film system.*

TABLE I—TABULATION OF PARTIAL MOLAR AREAS OF COMPONENT 2 IN MIXED LECITHIN FILMS

| Mixed Film System | Molecular Areas of Component 2, Å.² Partial Molar Areas in Mixed Film | at Collapse[a] | Extrapolated[a] to $\pi$ = 0 |
|---|---|---|---|
| Lecithin–oleic acid | 17 | 27.0 | 39 |
| Lecithin–elaidic acid | 8.7 | 26.0 | 35.6 |
| Lecithin–diglyceride acid | 19.8 | 39.5 | 47.5 |
| Lecithin–triglyceride acid | 33 | 59 | 65.8 |

[a] Averages of 2 or more values.

$N_2 \rightarrow 1$, the significance of these observations at low $N_2$ values remains clouded.

The lecithin–stearic acid and the lecithin–monoglyceride systems cannot be evaluated in the same manner, since the collapse pressures for stearic acid and monoglyceride films were both very similar to that of lecithin films. However, they do obey Eq. 1, which supports the hypothesis that these systems are either immiscible or miscible but devoid of any specific interaction.

## GENERAL DISCUSSION

The linear relationships existing between the extrapolated mean molecular area, the mean molecular area at the various $\pi$ values, and the collapse points *versus* the mole fraction of component 2 of the mixed films of the lecithin–stearic acid, the lecithin–monoglyceride systems—together with the fact that stearic acid, monoglyceride, and lecithin are similar types of compounds—would seem to indicate that these mixed films are miscible. However, they do not manifest a significant degree of interaction and as such may be considered to form ideal two-dimensional solutions.

The negative deviations from linearity seen in Figs. 7–10 for the lecithin–oleic acid and lecithin–elaidic acid would seem to indicate, along with the data in Table I, that these systems do form miscible films. They further show the existence of interactions between the lecithin molecules and those of oleic and elaidic acid. This is corroborated in the case of the lecithin–oleic acid system by Dervichian in a preliminary note (6), indicating that oleic acid condensed lecithin films of unknown purity by approximately 18%.

Interactions between film components similar to those seen at the air–water interface may also occur at the oil–water interface in emulsion systems. Where this is the case, we would expect better stabilizing effects for those film systems which manifest a significant degree of interaction. To some extent, this is supported by the work of Yeadon *et al.* (8), who found that purified lecithin mixed with oleic acid formed better emulsions than purified lecithin alone.

In the case of the lecithin–diglyceride and lecithin–triglyceride system, the plots of the extrapolated mean cross-sectional area and the mean cross-sectional area *versus* the mole fraction for component 2 appear to be linear indicating an ideal two-dimensional liquid. However, Figs. 13 and 14 would imply that di- and triglyceride both tend to cause a condensation of the lecithin film. Table I shows the partial molar areas for these compounds to be smaller than the extrapolated molecular cross-sectional area for either diglyceride or triglyceride alone, which indicates the presence of a significant amount of interaction between lecithin and the glycerides. Desnuelle (7), using phospholipids obtained by precipitation of their ether solutions into acetone and a crude extract of unsaturated triglyceride, showed that the unsaturated triglyceride caused a condensation of the phospholipid film. His work tends to corroborate the authors' findings with lecithin–triglyceride systems —although the triglyceride in our system contained saturated acyl groups.

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*Keyphrases*

Lecithin surface films
Soybean lecithin lipids-interaction of
Mean molecular area
Partial molar area
Film pressure
Collapse points