# Influence of Soybean Protein Isolates–Phosphatidycholine Interaction on the Stability of Oil-in-Water Emulsions

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**ABSTRACT:** Soybean protein isolates and phospholipids present specific surface properties with synergistic or antagonistic effects on emulsion stability. Oil-in-water emulsions (25:75 w/w) were prepared using native and denatured soybean isolates (NSI and DSI, respectively) with the addition of phosphatidylcholine (PC) (protein/PC ratio 100:1 to 10:1). The effect of ionic strength was also studied by adding sodium chloride (0–100 mM) to the aqueous phase. Analysis of NSI/PC and DSI/PC emulsions showed that the creaming rate diminished upon addition of PC, with the creamed phase showing more stability than those of the control systems. In DSI/PC systems, the coalescence process was partially controlled, as evidenced by a decrease in the size of oil droplets. Both systems were altered by the presence of sodium chloride, with an increase in the creaming rate attributable to flocculation and the coalescence of droplets. Under these conditions, DSI/PC emulsions exhibited a stronger protein–phospholipid interaction than those of NSI/PC.

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**KEY WORDS:** Oil-in-water (O/W) emulsion stability, phosphatidylcholine, protein–phospholipid interaction, soybean isolates, vertical scan analyzer.

Proteins and phospholipids are important nutritional components that contribute to the taste and texture of foods. Also, they have interesting surface properties that enable them to achieve emulsion stability in different products (1). Interactions between proteins and phospholipids may lead to changes in surface activity, modifications of protein structure, and incorporation of protein into surfactant micelles and vesicles (2).

Soy proteins have good functional properties for food processing (3). A study of the interaction between these proteins and phosphatidylcholine (PC) has confirmed the existence of a protein–lipid complex with different degrees of association for 7S and 11S globulin preparations (4). Nevertheless, further research is necessary to understand soy protein–phospholipid interactions and their effects on emulsion stability.

Emulsions are unstable systems from a physicochemical point of view, evolving from a homogeneous system at the beginning to complete phase separation. The physical destabilization mechanisms of emulsions include oil droplet size variation processes, such as flocculation and coalescence, and particle migration phenomena, such as sedimentation and creaming.

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Several emulsifiers are efficient at reducing the interfacial tension, with the characteristic of migrating to the oil–water interface according to their hydrophilic–lipophilic balance (5,6).

In food emulsions, some components are ionic, whereas others, including salts, proteins, and phospholipids, have the capacity to be ionized. Also, the ionic strength can affect the stability of emulsions by modifying the hydrodynamic interactions between oil droplets (7).

The objectives of the present work were (i) to study soy protein isolate–PC interactions and their effects on oil-in-water (O/W) emulsion stability, (ii) to evaluate the influence of protein structure, and (iii) to determine the effects of the emulsifier agent ratio and different ionic strengths.

## EXPERIMENTAL PROCEDURES

*Materials. (i) Soy proteins*. Native soy isolate (NSI) was prepared according to the method of Sorgentini and Wagner (8). Denatured soy isolate (DSI) was obtained by heating NSI dispersions at 90°C for 5 min. Dispersions of soy proteins were obtained in a 0.01 M sodium phosphate buffer, pH 7.0.

*(ii) Phospholipids*. Soybean PC with purity greater than 98% was purchased from Sigma Chemical Co. (St. Louis, MO).

*(iii) O/W emulsions*. Refined sunflower seed oil, provided by a local oil industry, was used for the formulation of emulsions. O/W emulsions (25:75 w/w) were prepared at room temperature in an Ultra-Turrax T25 homogenizer at a rate of 20,000 rpm for 30 s. Dispersions of NSI and DSI without (protein control) and with the addition of PC in protein/PC ratios ranging from 100:1 to 10:1 were used. Emulsion systems containing only PC (phospholipid control) in different ranges were also analyzed.

The effect of ionic strength was studied by adding NaCl ranging from 0–100 mM to the aqueous phase of different systems.

*Emulsion stability*. All dispersions were optically characterized using a vertical scan analyzer (QuickSCAN) as described previously (9). This equipment allows one to analyze a sample contained in a cylindrical glass measurement cell by a mobile reading head composed of a pulsed near-IR light source ($\lambda$ = 850 nm) and two synchronous detectors. The transmission detector was set at 0° and the backscattering detector was set at 135°, thus allowing scanning of the entire wavelength of the sample (approx. 65 mm) at a given time.

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It was possible to obtain curves of transmitted and backscattered light flux (in percent, relative to external standards) as a function of sample height (in mm). Results were expressed as backscattering (BS%), transmission (T%), or compared to a reference profile (time = 0 min), obtaining, in this case, the corresponding delta backscattering (ΔBS) and delta transmission (ΔT) profiles, respectively. In this way, it was possible to discriminate between particle migration (creaming) and particle size variation (flocculation, coalescence) processes.

Coalescence and creaming kinetics were observed by plotting the mean values of backscattering or transmission of peaks as a function of time in different zones of the sample.

Coalescence of emulsions was also evaluated under controlled stirring ($t$ = 0–60 min) by spectrophotometric techniques at λ = 500 nm (10).

*Particle size distribution.* Emulsions were analyzed using a Mastersizer Micro Particle Analyzer (Malvern Instruments Ltd.), which is a laser diffraction-based particle size analyzer. Oil droplet size distribution was determined by volume. Sauter mean diameter, $D[3,2]$, was calculated as follows:

$$D[m,n] = \left[ \frac{\sum_i V_i d_i^{m-3}}{\sum_i V_i d_i^{n-2}} \right]^{1/(m-n)} \quad [1]$$

where $V_i$ = relative volume in class $i$, $d_i$ = mean class diameter, $m$ = 3, and $n$ = 2.

## RESULTS AND DISCUSSION

Figure 1A shows the ΔBS profiles corresponding to the PC control system of an O/W emulsion obtained by the addition of only PC corresponding to a 25:1 buffer/PC ratio. A coalescence process (evidenced by a negative ΔBS) can be observed along the tube length of the sample. Similar results were obtained for PC control systems with other buffer/PC ratios assayed (100:1, 50:1, 10:1).

ΔBS profiles for NSI and DSI of O/W emulsions (protein control systems) can be seen in Figures 1B and 1C. The creaming process and creamed phase zones (I and II) were detected over the tube length in both cases. Important differences were recorded in each zone for both NSI and DSI emulsions.

The behavior of NSI/PC (10:1) and DSI/PC (10:1) systems also could be visualized by the evolution of ΔBS profiles (Figs. 1D and 1E), where the zones described previously were clearly distinguished.

The effect of PC on the creaming kinetics of NSI formulations was analyzed at the tube bottom (zone I, thickness 9–10 mm) (Fig. 2A). Emulsions prepared with the addition of PC were more stable against this destabilization process than the control system, suggesting a more efficient interaction at the lowest NSI/PC ratio assayed (10:1). Different BS% profiles of the creamed phase (zone II, thickness 45–50 mm) for NSI/PC emulsions can be observed in Figure 2B. When the amount of PC increased, the BS% evolved in the same way. A diminution of $D[3,2]$ of the particles with the addition of PC in the initial emulsion could not be observed (Table 1). The effect of PC on creaming kinetics and particle density of the creamed phase could be related to an increase in the interfacial charge, which would protect the emulsion against the flocculation process (11). Nevertheless, an increase was observed in oil droplet size in the creamed phase, compared to the initial stage, for the NSI control and NSI/PC systems: $D[3,2]$ = 23.5 and 17–19 μm, respectively. These results suggest that the presence of the phospholipid can bring about a partial control of coalescence.

The comparative evolution of creaming kinetics related to DSI/PC emulsions (zone I, thickness 24–26 mm) is shown in Figure 2C. At $t$ = 0, BS% values increased with the addition of increasing amounts of PC. BS% then remained constant over a longer period of time, especially when the highest amount of PC was used. These results would explain the diminution of particle size (see Table 1) and probably the inhibition of the flocculation process (7). Also, the creaming rate diminished in the presence of PC, significantly increasing the emulsion stability mainly for DSI/PC (10:1). These results suggest that DSI/PC emulsions have more effective protein–phospholipid interactions than those corresponding to NSI/PC systems. In a previous paper it was reported that DSI proteins have a surface hydrophobicity four times higher than NSI (10), which could promote hydrophobic interactions between the denatured proteins and phospholipids. These results are in agreement with those reported by other researchers (5). On the other hand, the creamed phase of DSI emulsions was not affected by the addition of PC (data not shown).

Evaluation of the coalescence process in NSI/PC and DSI/PC systems under controlled stirring as a function of time is shown in Figures 3A and 3B. Although stirring induced the destabilization of emulsions by coalescence by enhancing the probability of droplet collisions, NSI systems that included PC presented more stability against this mechanical effect. The protective role of PC was more evident when the NSI/PC ratio was reduced below 50:1, probably associated with the effective increase in the electrostatic repulsion of particles (7,11).

Figure 3B shows that the DSI emulsions, which coalesced more quickly than those of NSI under stirring, were not stabilized by the addition of PC. These results suggest that the DSI/PC interaction would not significantly affect the resistance of the interfacial film. In a previous paper it was reported that the destabilization by coalescence under this condition was enhanced by the addition of NaCl (10). The negative effect of ionic strength was not prevented by the presence of PC, as was observed for emulsions prepared with either NSI/PC or DSI/PC (Fig. 3C).

The effect of ionic strength on the creaming process of NSI/PC emulsions can be observed in Figures 4A and 4B. The ΔT and ΔBS profiles corresponding to an emulsion of NSI/PC (10:1) with 100 mM NaCl exhibited marked modifications compared with the control system, such as the appearance of an increase in ΔT and a distortion in ΔBS profiles. These changes could be related to the quick flocculation and creaming

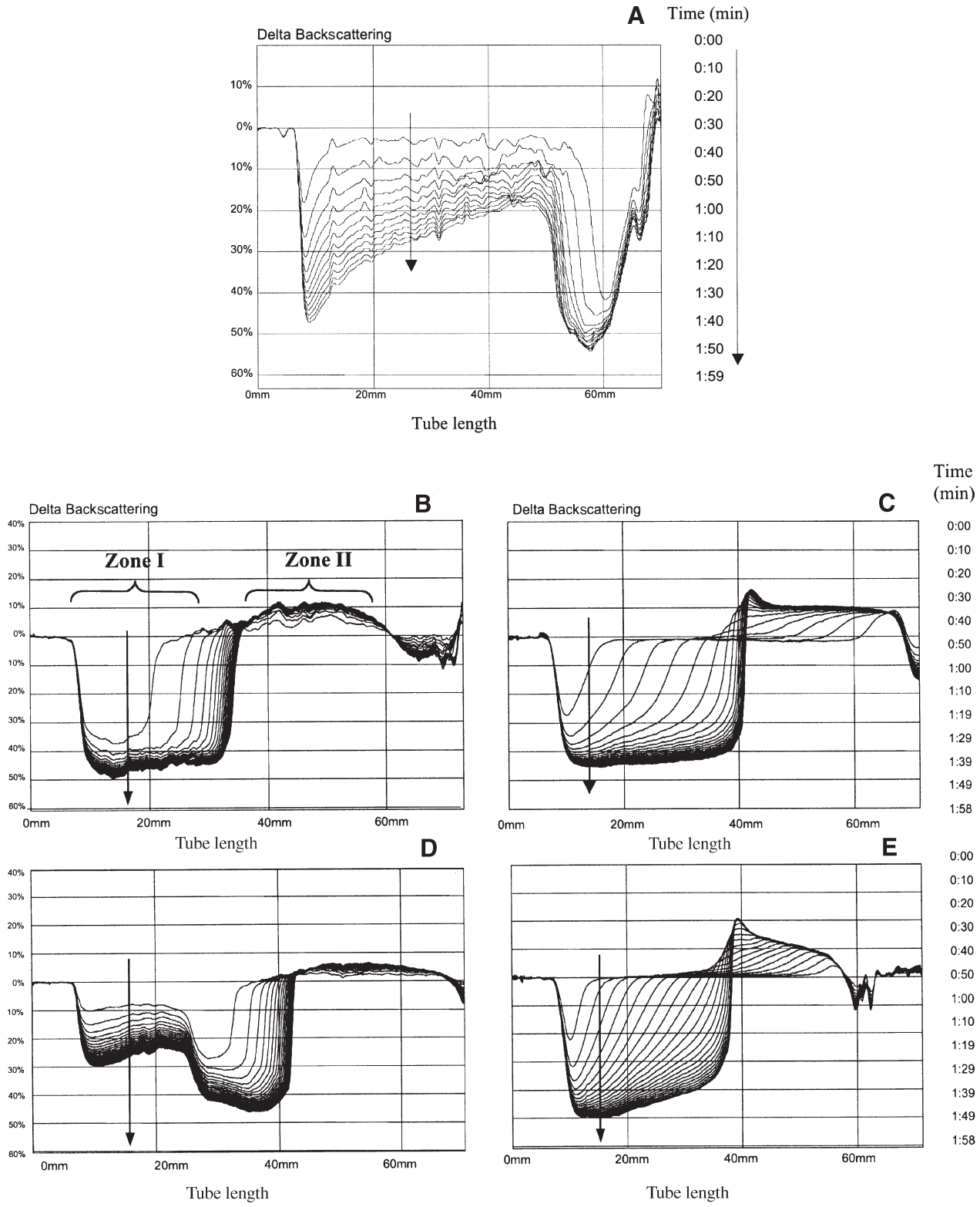


**FIG. 1.** Delta backscattering (ΔBS) profiles for oil-in-water (O/W) emulsions. (A) Formulated with the addition of only PC in a 25:1 ratio (phospholipid control system) or formulated with only soy proteins (protein control system); (B) native soy isolate (NSI); (C) denatured soy isolate (DSI) and with the addition of PC; (D) NSI/PC (10:1); (E) DSI/PC (10:1). Arrows denote time as represented on the right scale.

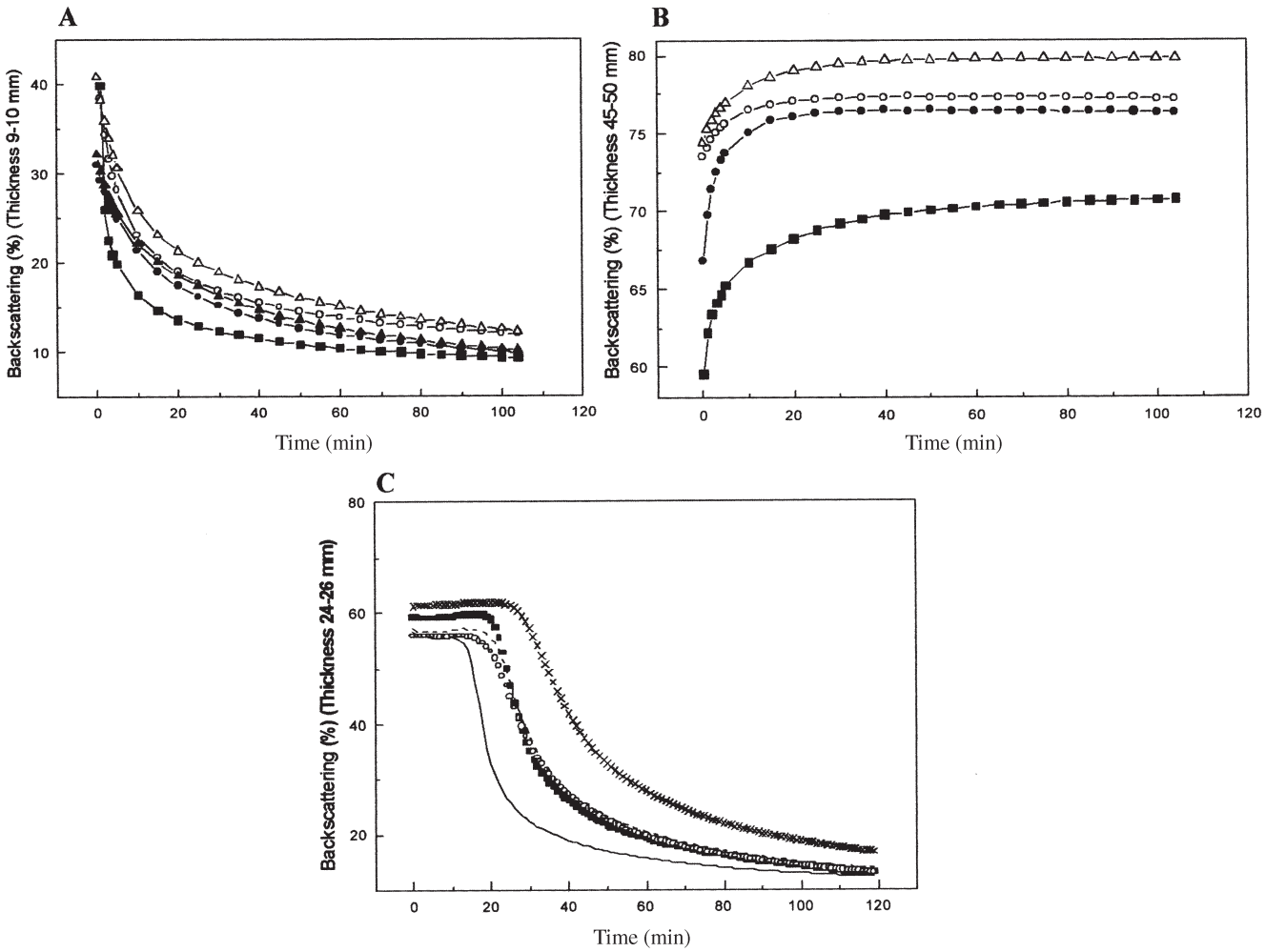


**FIG. 2.** O/W creaming destabilization kinetics (zone I, thickness 9–10 mm). (A) (■) NSI control system; (●) NSI/PC 100:1; (▲) NSI/PC 50:1; (○) NSI/PC 25:1; (△) NSI/PC 10:1. (B) Creamed phase (zone II, thickness 45–50 mm). (■) NSI control system; (●) NSI/PC 100:1; (○) NSI/PC 25:1; (△) NSI/PC 10:1. (C) O/W creaming destabilization kinetics (zone I, thickness 24–26 mm). (——) DSI control system; (– – –) DSI/PC 100:1; (■) DSI/PC 50:1; (○) DSI/PC 25:1; (×) DSI/PC 10:1. Plotted values are the means of two determinations. For abbreviations see Figure 1.

**TABLE 1**
**Sauter Mean Diameter (*D*[3,2]) of Oil Droplets in Native Soy Isolate (NSI)/PC and Denatured Soy Isolate (DSI)/PC Oil-in-Water (O/W) Emulsions with Different Protein/Phospholipid Ratios[a]**

| Sample | *D*[3,2] (μm) | Sample | *D*[3,2] (μm) |
|---|---|---|---|
| NSI control | 8.7 | DSI control | 12.2 |
| NSI/PC 100:1 | 9.1 | DSI/PC 100:1 | 12.2 |
| NSI/PC 50:1 | 8.9 | DSI/PC 50:1 | 11.6 |
| NSI/PC 25:1 | 8.8 | DSI/PC 25:1 | 11.3 |
| NSI/PC 10:1 | 11.6 | DSI/PC 10:1 | 10.5 |

[a]Maximum SD: 0.2 μm.

of droplets, achieving a clarification at the tube bottom. This process also can be accelerated by the coalescence induced by the effect of NaCl (Table 2).

The destabilization kinetics for NSI/PC systems brought about by the addition of NaCl was evident from an increase in transmission values, which were significant at levels of concentration of 75–100 mM (Fig. 4C). This behavior was similar to that observed in NSI emulsions formulated without PC (data not shown).

For DSI/PC/NaCl systems, the corresponding profiles were modified when compared to the corresponding control systems (see Fig. 1E), but the increase in ΔT values (Fig. 5A) was less important than those recorded for NSI/PC/NaCl systems (see Fig. 4A). Also, it was possible to observe modifications related

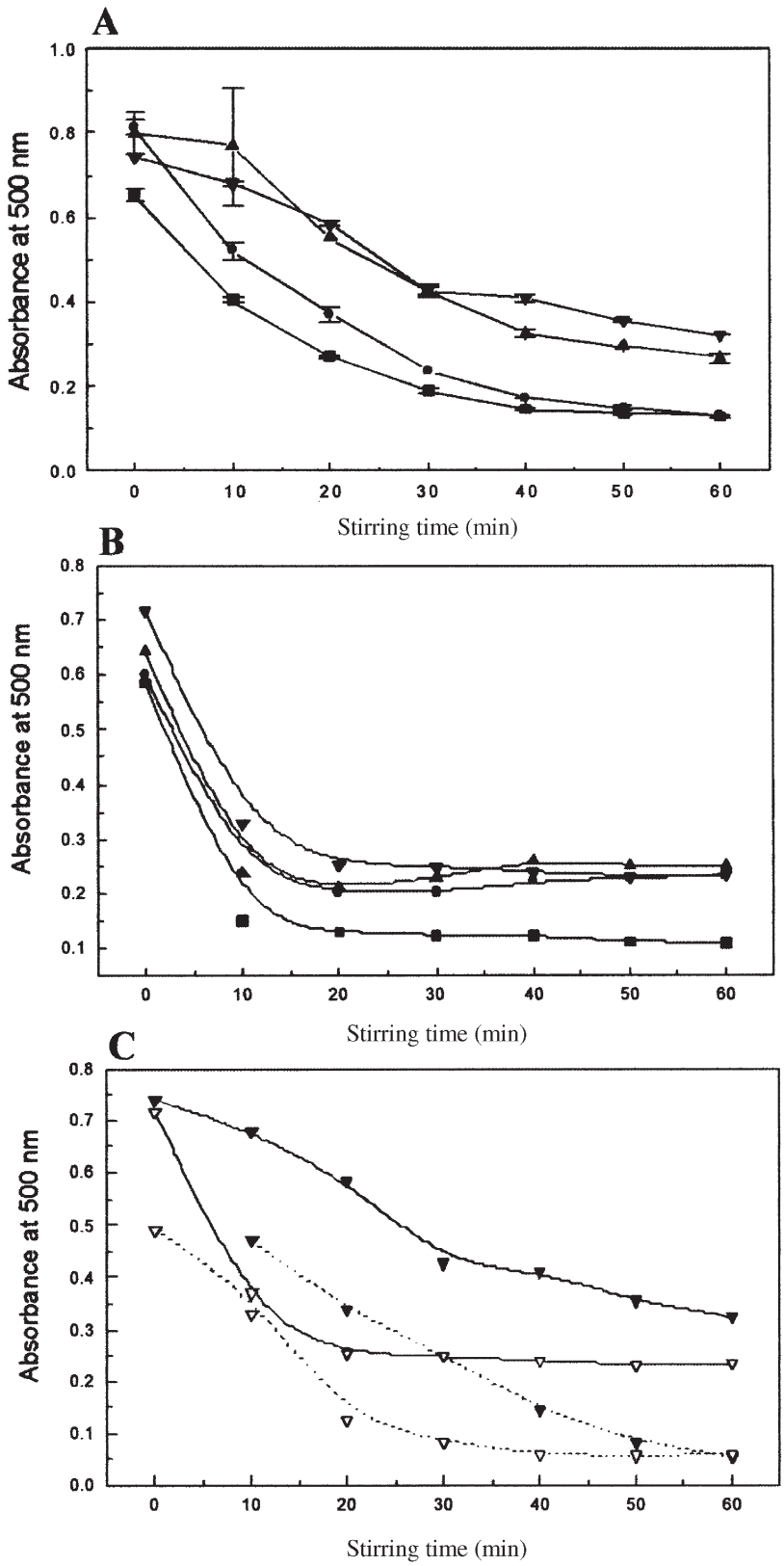


**FIG. 3.** Coalescence process under controlled stirring. (A) (■) NSI control system; (●) NSI/PC 50:1, (▲) NSI/PC 25:1; (▼) NSI/PC 10:1. (B) (■) DSI control system; (●) DSI/PC 50:1; (▲) DSI/PC 25:1; (▼) DSI/PC 10:1. (C): (▼) NSI/PC 10:1; (▽) DSI/PC 10:1; (——) without NaCl; (.....) with NaCl. Plotted values are the means of three determinations. Error bars represent SD. For abbreviations see Figure 1.

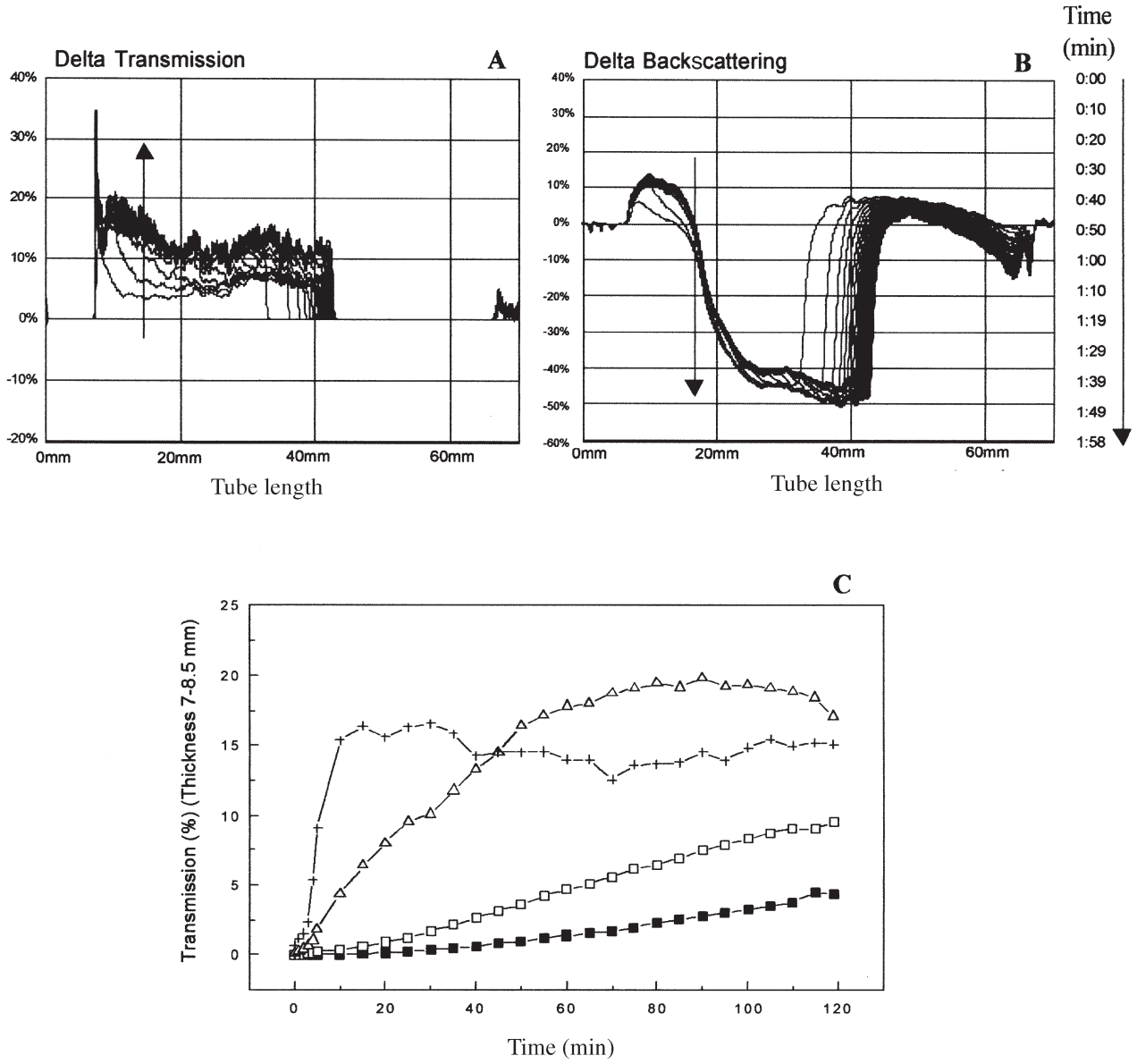


**FIG. 4.** Destabilization profiles for an O/W emulsion of NSI/PC (10:1) with 100 mM/NaCl. (A) NaCl Delta transmission (ΔT); (B) delta backscattering (ΔBS). Arrows denote time as represented on the right scale. (C) Destabilization kinetics by the addition of NaCl (transmission zone I, thickness 7–8.5 mm). (■) Without NaCl; (□) 40 mM NaCl; (△) 75 mM NaCl; (+) 100 mM NaCl. Plotted values are the means of two determinations. For abbreviations see Figure 1.

**TABLE 2**
**Effect of Ionic Strength on *D*[3,2] of Oil Droplets in NSI/PC and DSI/PC O/W Emulsions (protein/phospholipid ratio 10:1)[a]**

| Sample | *D*[3,2] (μm) | Sample | *D*[3,2] (μm) |
|---|---|---|---|
| NSI/PC 0 mM NaCl | 11.6 | DSI/PC 0 mM NaCl | 10.5 |
| NSI/PC 40mM NaCl | 21.3 | DSI/PC 40 mM NaCl | 11.0 |
| NSI/PC 75 mM NaCl | 23.0 | | |
| NSI/PC 100mM NaCl | 24.2 | DSI/PC 100 mM NaCl | 15.4 |

[a]Maximum SD: 0.2 μm. For abbreviations see Table 1.

to the evolution of the creaming process and the creamed phase (Fig. 5B). The comparative evolution of the BS% for DSI/NaCl systems with and without the addition of PC as a function of time is shown in Figures 5C and 5D, respectively. The effect of a strong protein–phospholipid interaction and less influence of NaCl for DSI/PC/NaCl emulsions, in which the addition of PC produced a significant decrease in the creaming rate as a consequence of a minor extension of the

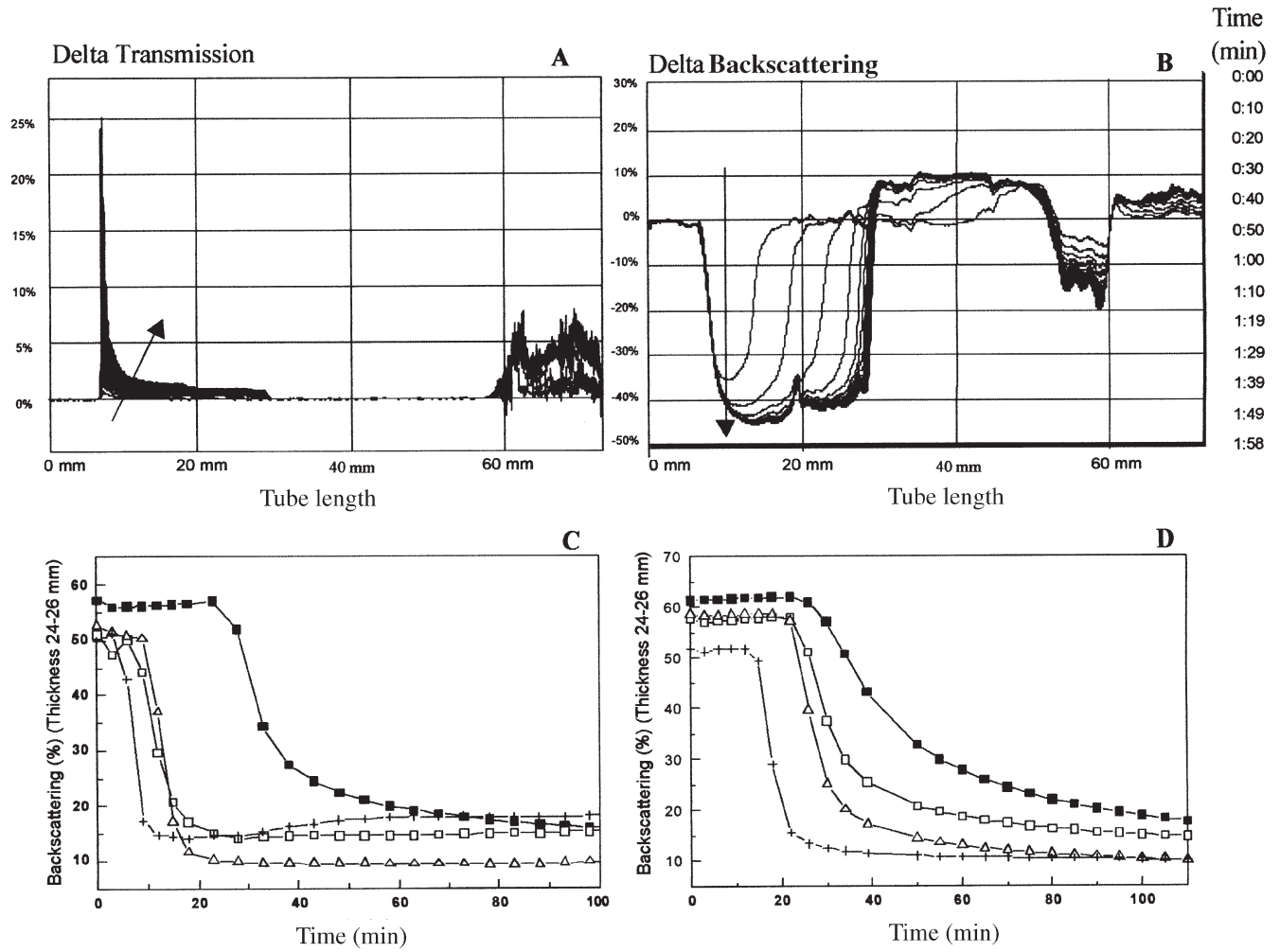


**FIG. 5.** Destabilization profiles for an O/W emulsion of DSI/PC (10:1) with 100 mM/NaCl. (A) ΔT; (B) ΔBS. Arrows denote time as represented on the right scale. Destabilization kinetics by the addition of NaCl (backscattering %, thickness 24–26 mm). (C) DSI emulsions (■) without NaCl, (□) with 40 mM NaCl, (△) with 75 mM NaCl, or (+) with 100 mM NaCl. (D) DSI/PC 10:1 emulsions (■) without NaCl, (□) with 40 mM NaCl, (△) with 75 mM NaCl, or (+) with 100 mM NaCl. Plotted values are the means of two determinations. For abbreviations see Figures 1 and 4.

coalescence process, must be noted. This information correlated with the results presented in Table 2.

In conclusion, soy protein isolates, in a native or denatured state, displayed different behavior as emulsifying agents, either alone or in interaction with PC. Also, the presence of PC diminished the creaming rate in both systems (NSI/PC and DSI/PC) and produced creamed phases with different physicochemical characteristics. Coalescence under controlled stirring decreased with an enhancement in the protein/PC ratio for NSI/PC. This effect was less pronounced in the case of DSI/PC emulsions. The destabilization effect of ionic strength was only partially controlled in DSI/PC systems.

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