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# Interfacial Rheological and Tension Properties of Protein Films

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**The purpose of this study was to investigate and compare interfacial rheological and tension properties of adsorbed protein films under conditions known to affect bulk properties of proteins. In previous publications, the effects of pH, protein concentration, temperature, and aging on the interfacial rheology of bovine serum albumin (BSA) and human immunoglobulin G ($HI_gG$) were reported (D. J. Burgess, L. Longo, and J. K. Yoon,** *J. Parenteral Sci. Technol.* **45, 239 (1991); and D. J. Burgess, J. K. Yoon, and N. O. Sahin,** *J. Parenteral Sci. Technol.* **46, 150 (1992)). These data are compared with interfacial tension data reported here. In addition, the effects of ionic strength and chemical agents on the interfacial rheology and tension of BSA and $HI_gG$ are reported. An oscillatory interfacial shear rheometer was used to determine interfacial rheology, and a Cahn microbalance connected to a Wilhelmy plate was used to measure interfacial tension. These two techniques provide information on molecular interfacial adsorption, interaction between adsorbed molecules, film compactness, and strength. They appear to be complementary when used in the characterization of adsorbed protein interfacial films.** © 1997 Academic Press

*Key Words:* **interfacial rheology; interfacial tension; protein films; albumin; human immunoglobulin G.**

## INTRODUCTION

Proteins are amphiphilic compounds composed of polar and nonpolar amino acid residues. Consequently, they adsorb at interfaces, rearranging to expose their hydrophobic residues to the hydrophobic phase and their hydrophilic residues to the aqueous phase (3). Protein interfacial adsorption leads to configurational changes which may result in loss of tertiary and quaternary structure and may be reversible or irreversible (3). Irreversible configurational changes which take place at the interface may lead to aggregation and precipitation should these molecules return to the bulk phase. For example, aggregation and precipitation in insulin solutions have been reported to occur following handling, such as shaking which temporarily increases the interfacial area as a result of the introduction of air bubbles (3). Configurational changes and physical instability of bulk protein molecules may result as consequences of environmental changes, including pH, ionic strength, temperature, aging, protein concentration, and the addition of certain chemical agents (3–5). Urea, guanidine hydrochloride, and copper sulfate are examples of protein chemical denaturants (5). These molecules bind to proteins, resulting in loss of tertiary and quaternary structure. pH affects the ionization of the carboxyl and amino groups of proteins, which in turn may affect intramolecular interaction and hence folding (3, 4). The effective charge on proteins is dependent on the ionic strength through counterion screening, and consequently ionic strength alters molecular folding. Change in the molecular thermal energy of proteins can result in conformational change (3), which can be reversible or irreversible, depending on the temperature. High temperatures can induce bond cleavage and hence irreversible denaturation. In the present study, interfacial tension and interfacial shear rheology are investigated to assess their usefulness in characterization of adsorbed protein films with respect to parameters known to affect protein properties in the bulk (protein concentration, pH, temperature, aging, and the addition of additives).

In a series of papers, Graham and Phillips (6–9) reported adsorption kinetics and surface denaturation of proteins at liquid interfaces. These authors investigated the effects of heat denaturation and pH on surface pressure and dilational viscosity of protein films. In previous publications the effects of pH, protein concentration, temperature, and aging on the interfacial rheology of bovine serum albumin (BSA) and human immunoglobulin G ($HI_gG$) were reported (1, 2). It was concluded that interfacial rheology may be useful as an indicator of protein configurational change, competition between molecules for interfacial space, and film strength. In the present study interfacial rheology and tension are compared for BSA and $HI_gG$. Interfacial tension data provide an indication of film compactness, the lower the interfacial tension, the more compact the film for comparable systems. Interfacial tension is investigated here using a modified Wilhelmy plate method. Interfacial rheology can be described as the resistance of the interface to deformation

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**TABLE 1**
**Effect of Bulk Concentration of BSA and $HI_gG$ on Interfacial Elasticity and Interfacial Tension**

| BSA | | $HI_gG$ | |
|---|---|---|---|
| Bulk concentration (% w/v) | Interfacial elasticity (mN/m) ± SD | Bulk concentration (% w/v) | Interfacial elasticity (mN/m) ± SD |
| 0.1 | 12.3 ± 0.8 | 0.75 | 196.3 ± 1.2 |
| 0.5 | 113.2 ± 0.9 | 1.5 | 1102.6 ± 2.7 |
| 1.0 | 237.0 ± 1.2 | | |
| 2.0 | 375.0 ± 2.5 | | |
| 4.0 | 564 ± 2.8 | | |

| Bulk concentration (% w/v) | Interfacial tension (dyn/cm ± SD) | |
|---|---|---|
| | BSA | $HI_gG$ |
| 0.000001 | 56.5 ± 0.03 | nd[a] |
| 0.00001 | 54.8 ± 0.02 | nd[a] |
| 0.0001 | 51.1 ± 0.02 | 57.2 ± 0.03 |
| 0.001 | 46.4 ± 0.03 | 54.1 ± 0.02 |
| 0.01 | 46.2 ± 0.02 | 53.8 ± 0.03 |
| 0.1 | 46.1 ± 0.02 | 53.0 ± 0.03 |
| 1.0 | 45.2 ± 0.03 | 50.2 ± 0.02 |

*Note.* BSA, ionic strength = 100 m*M*, pH 7.4, 25°C. $HI_gG$, ionic strength = 100 m*M*, pH 5.5, 25°C. $n = 5$ (interfacial elasticity) and $n = 3$ (interfacial tension).

[a] Not determined.

and is a measure of film strength (1, 2, 10–12). This is a nondestructive method, involving continuous monitoring of the interface. An oscillatory ring interfacial rheometer, operating in the shear mode, is used to determine interfacial elasticity. This technique is nondestructive of the interface, as there is no alteration in interfacial area during measurement and therefore no movement of molecules into or out of the interface.

BSA is a globular protein with a molecular weight of 66,338 Da (13). It is a single-chain protein consisting of approximately 585 amino acid residues with three domains. It is intrinsically flexible, with 17 disulfide bonds which create nine double loops, and is approximately 50% $\alpha$-helix in the native state (14, 15). Most of the hydrophobic residues are between the subdomains, and the polar residues are located on the outer surfaces. The domains are different with respect to hydrophobicity, net charge, and ligand sites. BSA undergoes denaturation in the presence of urea, guanidine hydrochloride, and specific ions.

$HI_gG$ has a molecular weight of 150,000 Da. It has a Y-shaped structure and is composed of two heavy and two light chains held together by disulfide bonds (16). The most predominant feature of the tertiary structure of immunoglobulins is the immunoglobulin fold, which is a structural motif consisting of two stacked $\beta$-sheets surrounding an interior packed with hydrophobic amino acid residues (16, 17).

## MATERIALS AND METHODS

BSA (purified and essentially fatty acid free), ethylenediaminetetraacetic acid (EDTA), urea, guanidine hydrochloride, acacia, dextran, dextran sulfate, and calcium chloride were obtained from Sigma Chemical Co. Purified, lyophilized $HI_gG$ was obtained from ICN Biochemicals, Inc. All other chemicals were of analytical grade and were obtained from Fisher Scientific. Single distilled, deionized water was filtered through a series of filters arranged in the following order: carbon, anion and cation exchange, and organic. Ultrapurified water was obtained by redistillation of the filtered, single-distilled, deionized water from acidic potassium permanganate solution, using an all-glass still. Ultrapurified water was used in the preparation of all aqueous solutions. It was stored prior to use in capped airtight bottles and used within 24 h.

BSA and $HI_gG$ were analyzed chromatographically using a Sephadex G-25 column to check for purity and the presence of dimers, trimers, and any larger aggregated units. Concentrated aqueous solutions of BSA and $HI_gG$ were prepared

**TABLE 2**
**Effect of Temperature on the Interfacial Elasticity and Tension of BSA and $HI_gG$**

| | BSA | | $HI_gG$ | |
|---|---|---|---|---|
| Temperature (°C) | Interfacial elasticity (mN/m) ± SD | Interfacial tension (dyn/cm) ± SD | Interfacial elasticity (mN/m) + SD | Interfacial tension (dyn/cm) ± SD |
| 25 | 237.0 ± 1.2 | 45.2 ± 0.03 | 1102.6 ± 2.7 | 50.2 ± 0.02[a] |
| 37 | 89.7 ± 0.8 | 47.0 ± 0.02 | 200.5 ± 1.2 | nd[b] |
| 40 | 42.5 ± 0.7 | nd[b] | nd[b] | nd[b] |
| 60 | 31.2 ± 1.2 | nd[b] | nd[b] | nd[b] |

*Note.* BSA, ionic strength = 100 m*M*, pH 7.4, 1% w/v. $HI_gG$, ionic strength = 100 m*M*, pH 5.5, 1.5% w/v. $n = 5$ (interfacial elasticity) and $n = 3$ (interfacial tension).

[a] Concentration was 1% w/v.

[b] Not determined.

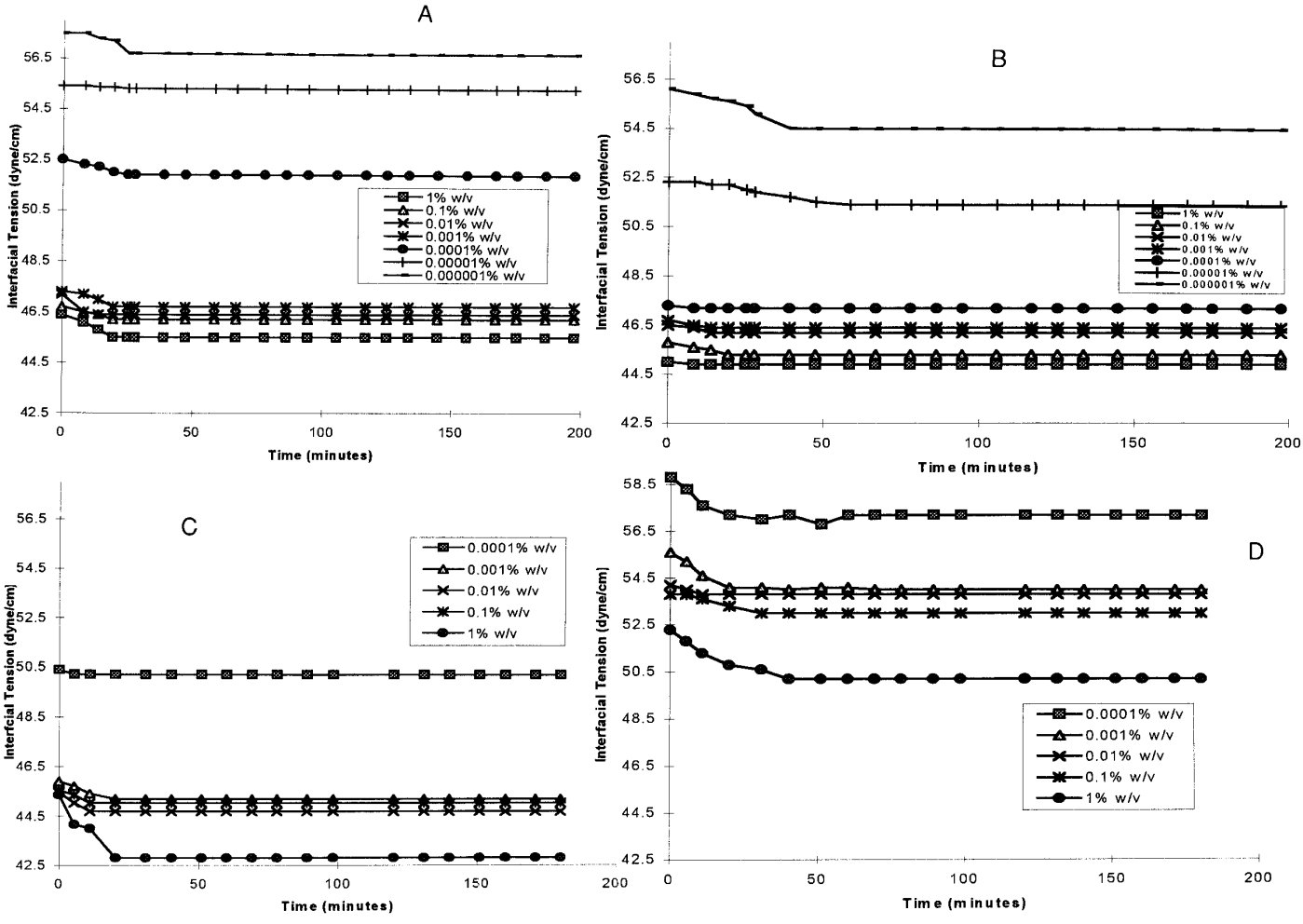


**FIG. 1.** The effect of bulk concentration on the interfacial tension of BSA (100 m*M*, 25°C) at (A) pH 7.4 and (B) pH 5.5, and of $HI_gG$ (100 m*M*, 25°C) at (C) pH 7.4 and (D) pH 5.5 at the air/aqueous interface. The values are the mean of three measurements. The error bars indicate ± range. $n = 3$.

and the pH was adjusted to pH 5.5 and 7.4, respectively, using 10 m*M* solutions of NaCl, HCl, and NaOH. These solutions were passed through Sephadex G-25 columns in 50 m*M* NaCl at a flow rate of 40 to 60 ml per hour and separation was monitored spectroscopically. Both proteins were pure and no aggregates were present.

*Preparation of Solutions*

All protein solutions were prepared in phosphate buffer in the pH range 3.4 to 8.0. Isotonicity was adjusted using the sodium chloride equivalent method. Ionic strength was maintained at 100 m*M* for all solutions other than those in which the ionic strength was varied (1–1000 m*M*). Concentrations in the range 1 to 4.0% w/v were prepared at 25 and 60°C, with minimal stirring to avoid foaming and subsequent protein denaturation. Solution preparation time was standardized at 1 h to equilibrate the solutions at the final temperature and to avoid error due to time-dependent protein denaturation. All solutions were used immediately. Samples were siphoned from under the air/water interface using a glass pipette.

*Interfacial Rheology*

A Mark II surface rheometer was used (Surface Science Enterprises, UK). The instrument consists of four systems: system I is a moving coil galvanometer; system II is a platinum du Nouy ring, which is placed at the interface and is attached to a galvanometer; system III is the surface rheometer control unit, which varies the driving frequency and monitors the amplitude of motion of the ring; system IV is an IBM PC, which drives the instrument and analyzes the data. The equation of motion for the apparatus and the associated theory are explained by Sherriff and Warburton (18). The experimental technique consists of positioning a platinum du Nouy ring at the interface, either gas/liquid or liquid/liquid. The ring oscillates through a few degrees

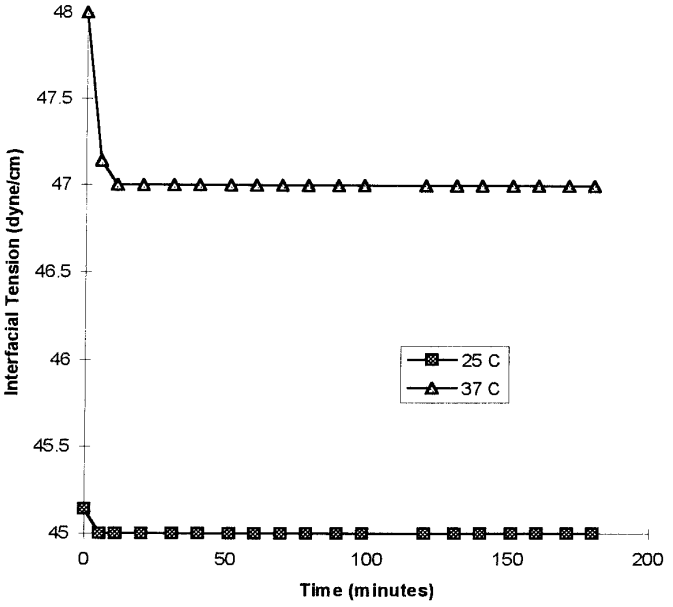


**FIG. 2.** The effect of temperature on the interfacial tension of BSA at the air/aqueous interface (100 m*M*, 1% w/v, pH 7.4). The values are the mean of three measurements. The error bars indicate ± range. $n = 3$.

about a vertical axis. The amplitude of motion of the ring is measured by a proximity probe transducer, and automatic analysis of the signal generated gives the dynamic surface rigidity modulus (surface elasticity, $G_s'$). $G_s'$ (mN/m) is defined as

$$G_s' = g_f \cdot I \cdot 4\pi(f^2 - f_0^2), \qquad [1]$$

where $I$ is the moment of inertia, $f$ is the sample interfacial resonance frequency, $f_0$ is the reference interfacial resonance frequency, and $g_f$ is the geometric factor. $g_f$ is defined as

$$g_f = 4\pi(R_1^2R_2^2)/[(R_1 + R_2)(R_2 - R_1)], \qquad [2]$$

where $R_1$ is the radius of the ring and $R_2$ is the radius of the sample cell.

Measurements were made with respect to a reference interface. The air/ultrapurified water interface was used as a reference for all experiments at the air/aqueous interface. Calibration against ultrapurified water was carried out prior to each measurement. Samples were filled into water-jacketed sample cells of internal diameter 3.853 cm. The temperature was maintained at the measurement temperature ±0.1°C. The du Nouy ring was lowered to sit at the interface. In all studies, the interface was broken prior to each measurement by the action of pouring into the sample cell and sweeping the surface with a clean glass rod. This removes any existing interfacial film, allowing the induction of a new film. The frequency of oscillation was always the resonant frequency. All measurements were repeated three times and the means and standard deviations determined.

It was shown previously that adsorbed films of BSA and $HI_gG$ exhibit elastic (solid-like) interfacial activity rather than viscous (liquid-like) activity (1, 2). Therefore interfacial elasticity values are reported here.

### *Interfacial Tension*

The apparatus consists of a Cahn 2000 recording microbalance sensor and control unit (Cahn Instruments, Inc., Cerritos, CA), a water-jacketed sample chamber, a circulating water bath with digital temperature control, a thermocouple for the sample chamber, a platinum Wilhelmy plate, an IBM PC-compatible data acquisition computer interfaced with an analog-to-digital converter, and a chart recorder. The temperature of the sample chamber was controlled to ±0.1°C using the circulating water bath and monitored using a thermocouple sensitive to ±0.1°C. The humidity over the sample chamber was maintained at 68 ± 2% during the experiments.

The aqueous phase was equilibrated at the predetermined temperature. The sample cell was raised slowly upward to

**TABLE 3**
**Effect of pH on the Interfacial Elasticity and Tension of BSA and $HI_gG$**

| | BSA | | | $HI_gG$ | |
|---|---|---|---|---|---|
| pH | Interfacial elasticity (mN/m) ± SD | Interfacial tension (dyn/cm) ± SD | pH | Interfacial elasticity (mN/m) ± SD | Interfacial tension (dyn/cm) ± SD |
| 3.0 | 152.6 ± 1.4 | nd[a] | 3.0 | 825.0 ± 1.2 | nd[a] |
| 5.0 | 97.0 ± 1.2 | 44.8 ± 0.02[b] | 5.5 | 1102.6 ± 2.7 | 50.2 ± 0.02 |
| 6.0 | 97.0 ± 1.2 | nd[a] | 7.4 | 155.0 ± 1.8 | 42.8 ± 0.02 |
| 7.4 | 564 ± 2.8 | 45.2 ± 0.03 | | | |

*Note.* BSA, ionic strength = 100 m*M*. 25°C, 4% w/v (elasticity data) and 1% w/v (tension data). $HI_gG$, ionic strength = 100 m*M*, 25°C, 1.5% w/v (elasticity data) and 1% w/v (tension data). $n = 5$ (interfacial elasticity) and $n =$ (interfacial tension).

[a] Not determined.

[b] pH was 5.5.

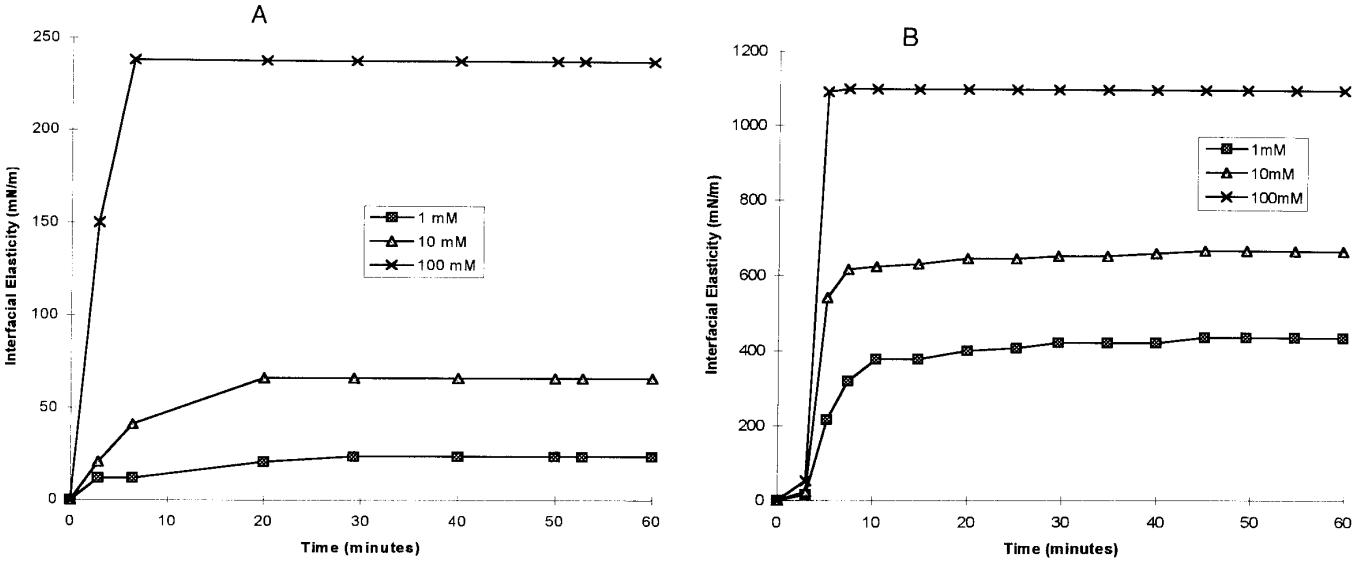


**FIG. 3.** The effect of ionic strength on the interfacial elasticity of (A) BSA (1% w/v, pH 7.4, 25°C) and $HI_gG$ (1.5% w/v, 25°C, pH 5.5) at the air/aqueous interface. The values are the mean of five measurements. The error bars indicate ± range. $n = 5$.

the plate using a vibration-free motorized labstand connected to a variable voltage source until the entire plate was submerged. The sample cell was lowered until the plate was completely above the interface and then raised slowly until the aqueous phase was attracted to the plate, forming a meniscus. The last step reproducibly placed the bottom edge of the plate exactly at the interface, and hence, error due to inconsistency of placement was eliminated.

The pulling tension at the interface was continuously monitored using the Cahn 2000 microbalance and recorded by the computer. Interfacial tension was calculated from the pulling tension, the contact angle, and the plate parameter using the equation

$$\gamma = \frac{p}{2(L + d)\cos\theta}, \qquad [3]$$

where $\gamma$ is the interfacial tension, $p$ is the interfacial pulling force, $L$ is the width of the plate, $d$ is the thickness of the plate, and $\theta$ is the interfacial contact angle. The interfacial contact angles were measured by the dynamic contact angle method reported previously (19). Interfacial tension measurements were carried out in triplicate and the mean values plotted.

Sandblasted glass plates were used. These plates were cleaned by washing in ultrapurified water five times, dipping in cleaning solution (1:4 v/v mixture of ethanol and hexane) for approximately 30 min to wash off emulsifier residues, soaking in sulfuric acid containing Nochromix for 30 min, and finally repeating the first step to wash off the cleaning solution.

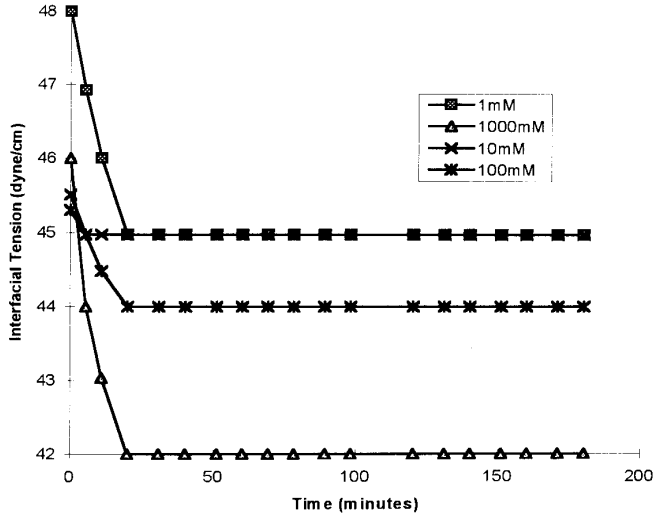


**FIG. 4.** The effect of ionic strength on the interfacial tension of BSA at the air/aqueous interface (100 m*M*, 1% w/v, pH 7.4). The values are the mean of three measurements. The error bars indicate ± range. $n = 3$.

## RESULTS AND DISCUSSION

### *Effects of Bulk Concentration and Temperature on Interfacial Properties*

The effects of bulk concentration and temperature on the interfacial elasticity of BSA and $HI_gG$ were reported in previous publications (1, 2). These data are summarized in Tables 1 and 2 for comparison with the equivalent interfacial tension data. The data reported in all tables are the interfacial elasticity and tension values at 60 min. Interfacial elasticity

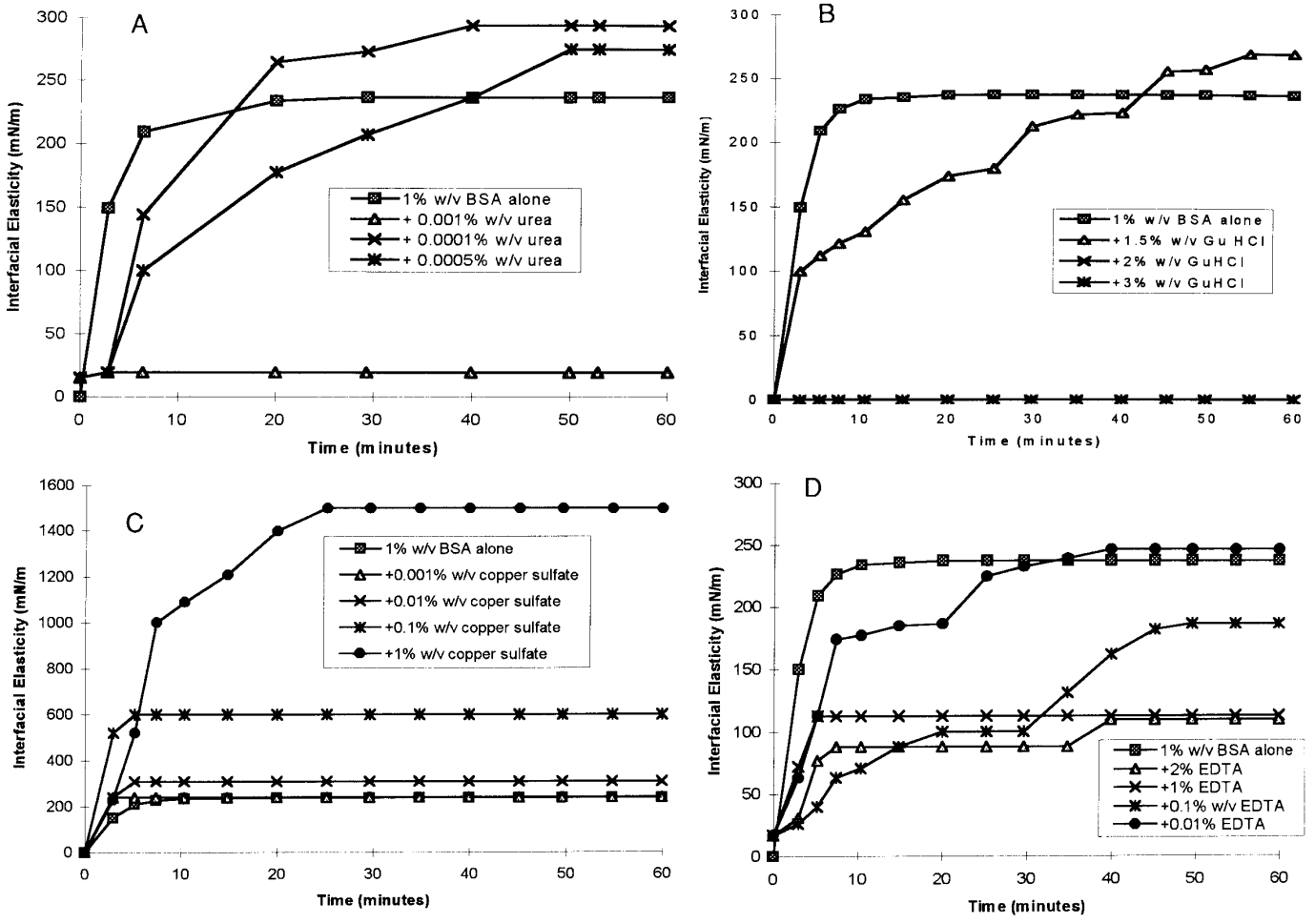


**FIG. 5.** The effect of the addition of (A) urea, (B) guanidine hydrochloride (GuHCl), (C) copper sulfate, and (D) EDTA on the interfacial elasticity of BSA at the air/aqueous interface (100 m*M*, 1% w/v, 25°C, pH 7.4). The values are the mean of five measurements. The error bars indicate ± range. $n = 5$.

increased with time in all cases (1, 2), whereas interfacial tension decreased with time, reaching equilibrium by 30 min (Figs. 1–8). The interfacial elasticities of BSA and $HI_gG$ increased with increased bulk protein concentration and decreased with increased temperature (Tables 1 and 2). For example, the interfacial elasticity of BSA (pH 7.4, ionic strength 100 m*M*, 25°C, 60 min) increased from 12.3 ± 0.8 to 564 ± 2.8 mN/m as the bulk concentration increased from 0.1 to 4% w/v. The interfacial elasticity of BSA (1% w/v, pH 7.4, ionic strength 100 m*M*, 60 min) decreased from 237.0 ± 1.2 to 31.2 ± 1.2 mN/m with an increase in temperature from 25 to 60°C. On the other hand, interfacial tension decreased with increased bulk concentration and increased with increased temperature (Figs. 1 and 2). The equilibrium interfacial tension of BSA solutions (100 m*M* and 25°C, 60 min) decreased from 56.5 ± 0.03 to 45.2 ± 0.03 dyn/cm and from 54.1 ± 0.03 to 44.8 ± 0.02 dyn/cm as the bulk concentration increased from $10^{-6}$ to 1% w/v, at pH 7.4 and 5.5, respectively. The interfacial tension of $HI_gG$ also decreased as bulk concentration increased. The equilibrium interfacial tension value of BSA at pH 7.4 increased from 45.2 ± 0.03 to 47.0 ± 0.02 dyn/cm as the temperature was raised from 25 to 37°C (Fig. 2).

### *Effects of pH and Ionic Strength on Interfacial Properties*

Interfacial elasticity and tension were at a minimum at the isoelectric pH values of BSA (pH 5.3) and $HI_gG$ (pH 7.4) under the conditions measured (BSA, 4% w/v, ionic strength 100 m*M*, 25°C; $HI_gG$, 1.5% w/v and 100 m*M*, 25°C) (Table 3, Fig. 1). An increase in ionic strength resulted in an increase in the interfacial elasticity of BSA and $HI_gG$ (Fig. 3) and a reduction in the interfacial tension of BSA (Fig. 4). The interfacial elasticities of BSA (1% w/v, pH 7.4, 25°C, 60 min) and $HI_gG$ (1.5% w/v, pH 5.5, 25°C, 60 min) increased from 18.6 ± 0.8 to 237.0 ± 1.2 mN/m and from 437.4 ± 1.8 to 1102.6 ± 2.7 mN/m, respectively, as ionic strength increased from 1 to 100 m*M*. No interfacial elasticity was

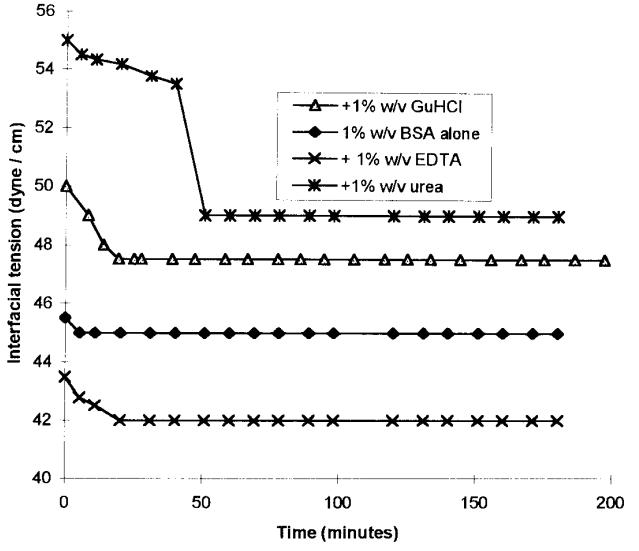


**FIG. 6.** The effect of the addition of 1% w/v urea, 1% w/v guanidine hydrochloride (GuHCl), and 1% w/v EDTA on the interfacial tension of BSA at the air/aqueous interface (100 m*M*, 1% w/v, 25°C, pH 7.4). The values are the mean of three measurements. The error bars indicate ± range. $n = 3$.

detected below 1 m*M* for both BSA and $HI_gG$. The interfacial tension of BSA decreased from 44.9 ± 0.02 to 41.8 ± 0.03 dyn/cm at pH 5.5 (close to the isoelectric pH value of BSA), as ionic strength increased from 1 to 1000 m*M*.

### *Effects of Additives on Interfacial Properties*

The interfacial properties of BSA were affected by the addition of denaturing and chelating agents (Figs. 5 and 6). Urea, guanidine hydrochloride, and EDTA resulted in an initial increase followed by a decrease in interfacial elasticity as the concentration of the denaturant was increased. Urea and guanidine hydrochloride caused an increase in interfacial tension, whereas EDTA reduced interfacial tension. The interfacial elasticity of BSA (1% w/v, pH 7.4, 100 m*M* NaCl, 25°C, 60 min) increased to 294.2 ± 1.2 mN/m in the presence of 0.0001% w/v urea and decreased to 27.6 ± 0.8 mN/m at 0.001% w/v urea. No interfacial elasticity was detected at the isoelectric pH of BSA after the addition of urea, even at low concentrations. The interfacial tension of BSA (1% w/v, pH 7.4, 100 m*M* at 25°C, 60 min) increased from 45.2 ± 0.03 to 49.0 ± 0.08 dyn/cm in the presence of 1% w/v urea. An initial slight increase in the elasticity of BSA with the addition of guanidine hydrochloride (1% w/v, pH 7.4, 100 m*M* NaCl, 25°C, 60 min) was followed by a reduction at higher concentrations, reaching zero at 3% w/v. The interfacial elasticity of BSA (1% w/v, pH 7.4, 100 m*M* NaCl, 25°C, 60 min) increased with the addition of copper sulfate (0.01 to 2.0% w/v) from 237.0 ± 1.2 to 1514.3 ± 2.6 mN/m.

The interfacial elasticity and tension of BSA were affected by the addition of the polysaccharides acacia, dextran, and dextran sulfate (Figs. 7 and 8). The addition of acacia (1% w/v) to BSA (1% w/v, pH 7.4, ionic strength 100 m*M*, 25°C, 60 min) resulted in a decrease in interfacial elasticity from 237.0 ± 1.2 to 85.2 ± 0.9 mN/m. However, acacia alone resulted in a strong elastic film (535.4 ± 2.2 mN/m). The addition of dextran decreased the interfacial elasticity of BSA (1% w/v, pH 7.4, ionic strength 100 m*M*, 25°C, 60 min) from 237.0 ± 1.2 to 95.2 ± 0.8 mN/m, whereas the addition of dextran sulfate resulted in a slight increase in interfacial elasticity to 275.3 ± 1.8 mN/m (Fig. 7). No interfacial elasticity was detected for solutions of dextran and dextran sulfate alone. The addition of acacia and dextran increased the interfacial tension of BSA (1% w/v, pH 7.4,

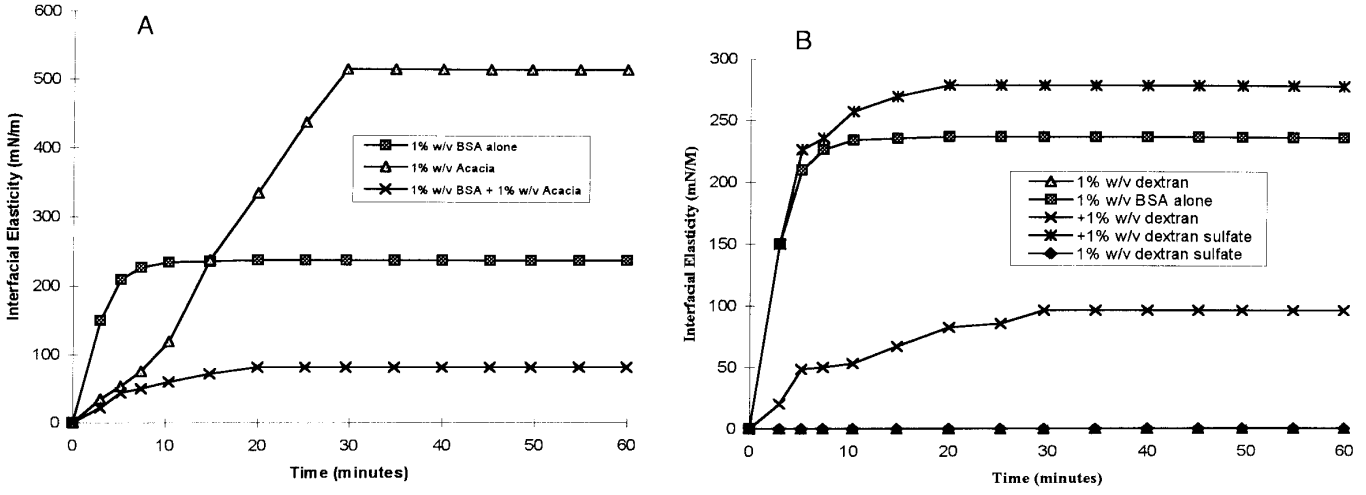


**FIG. 7.** The effect of the addition of (A) 1% w/v acacia and (B) 1% w/v dextran and dextran sulfate on the interfacial elasticity of BSA at the air/aqueous interface (100 m*M*, 1% w/v, 25°C, pH 7.4). The values are the mean of five measurements. The error bars indicate ± range. $n = 5$.

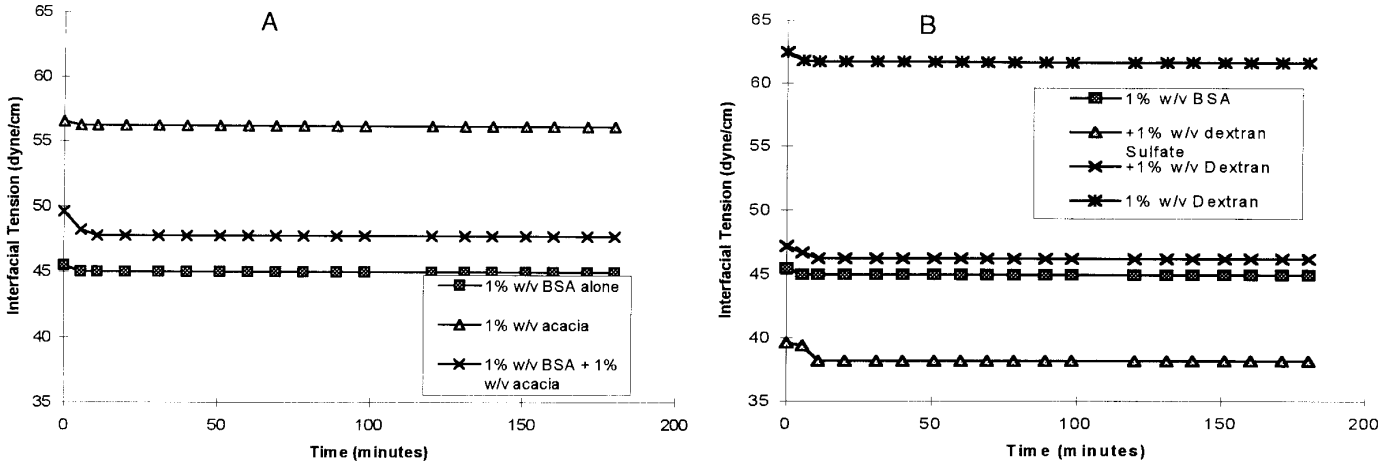


**FIG. 8.** The effect of the addition of (A) 1% w/v acacia and (B) 1% w/v dextran and dextran sulfate on the interfacial tension of BSA at the air/aqueous interface (100 m*M*, 1% w/v, 25°C, pH 7.4). The values are the mean of three measurements. The error bars indicate ± range. $n = 3$.

100 m*M* at 25°C, 60 min) from 45.2 ± 0.03 to 47.8 ± 0.02 dyn/cm and 45.2 ± 0.03 to 46.2 ± 0.05 dyn/cm, respectively. The addition of dextran sulfate decreased the interfacial tension of BSA (1% w/v, pH 7.4, 100 m*M* at 25°C, 60 min) from 45.2 ± 0.03 to 38.4 ± 0.03 dyn/cm.

## DISCUSSION

### *Effects of Bulk Concentration and Temperature on Interfacial Properties*

The increase in interfacial rheology of the protein solutions with time is considered to be a result of protein adsorption from the subsurface into the interface, molecular configurational change at the interface, intermolecular interaction, and the formation of multilayers (1, 2). When more molecules are adsorbed at the interface, the probability of entanglement between neighboring molecules is increased and consequently film strength, as measured by interfacial rheology, is higher. The reduction in interfacial tension with time is a result of protein adsorption and the ability of adsorbed protein to interact with both phases. The effects of temperature and bulk concentration on the interfacial properties of the protein solutions are related to interfacial adsorption; the higher the bulk concentration, the greater the interfacial adsorption, and the higher the molecular kinetic energy, the lower the interfacial adsorption. Additionally, the higher molecular kinetic energy results in a more fluid, less compact interfacial film.

### *Effects of pH and Ionic Strength on Interfacial Properties*

The interfacial elasticity and tension data appear to be in disagreement with respect to the effect of pH. The lower interfacial rheology data at the isoelectric pH values indicate a reduction in interfacial adsorption and/or interaction between molecules, whereas the lower interfacial tension data indicate an increase in interfacial adsorption. However, these results can be explained with consideration of molecular configuration. Proteins are in their most compact configuration at their isoelectric pH and therefore more molecules are able to adsorb at the interface, resulting in lower interfacial tension values. These compact structures are less likely to interact with one another compared to extended configurations which result at other pH values and, as a consequence, film strength (measured by interfacial elasticity) is decreased. From these results it appears that the tension data are dependent on interfacial adsorption and the rheology data are dependent on both interfacial interaction and adsorption.

The interfacial tension and rheology data complement each other with respect to the effect of ionic strength, since both indicate increased interfacial adsorption and/or increased lateral interaction between adsorbed molecules. An increase in ionic strength reduces the effective charge on the protein molecules as a consequence of counterion screening. At very low ionic strength (below 1 m*M*) it appears that the charges on the two proteins are sufficiently high that intermolecular electrostatic repulsion inhibits lateral interactions and hence interfacial elasticity. The tension data indicate that interfacial adsorption does occur under these ionic strength conditions although the amount adsorbed is reduced. As ionic strength is increased, electrostatic repulsion between neighboring molecules is reduced and consequently adsorption and lateral interactions increase. The increase in interfacial adsorption with an increase in ionic strength may also be a result of salting out, as the microions compete with the protein for water of hydration. Another possible explanation is that the shape of the protein molecules is changed due to counterion screening, resulting in a compact coiled conformation which can pack more easily at the interface. This explanation does not hold for the interfacial rheol-

ogy data, as compact configurations reduce lateral interactions and consequently interfacial rheology.

*Effects of Additives on Interfacial Properties*

Urea, guanidine hydrochloride, and EDTA are protein denaturants and act by disrupting hydrogen bonding, with characteristic changes in optical rotation, circular dichroism, and UV spectra (5). Low concentrations result in a slight unfolding of the protein molecules, which may explain the initial increase in interfacial elasticity. At high concentrations protein denaturation and precipitation occur. The absence of interfacial elasticity at the isoelectric pH of BSA, in the presence of urea, may be a consequence of the already low interfacial elasticity at this pH. Copper sulfate can form chelates with proteins, resulting in stronger more physically stable interfacial films, as is apparent from the interfacial elasticity data. The reduction in the interfacial tension of BSA in the presence of EDTA may also be a consequence of the formation of chelates, although this was not apparent from the interfacial elasticity data.

Acacia consists of a mixture of highly branched heteropolysaccharide molecules, such as arabinolactan (20), and contains bound protein. The protein–polysaccharide complex present in acacia can interact with proteins, trapping them in the core of the complex (20). The solubility of the resulting complex is low and consequently interfacial adsorption and elasticity are likely to be reduced and interfacial tension increased. Precipitation was evident in these samples, suggesting that this low solubility complex had formed. The increased interfacial elasticity and reduced interfacial tension of BSA in the presence of dextran sulfate are probably results of the formation of soluble electrostatic complexes. Dextran on the other hand does not complex with BSA and the reduction in interfacial activity with the addition of dextran may be a consequence of competitive adsorption between BSA and dextran. The rheology and tension data are in agreement with respect to the effects of polysaccharides on BSA interfacial properties.

## SUMMARY

Changes in the interfacial rheology and tension data of the proteins were related to interfacial adsorption, desorption, interaction, and configurational changes. The interfacial elasticity and tension values of the proteins investigated were time dependent and responded to changes in pH and ionic strength and the addition of chemical denaturants, chelating agents, and polysaccharides. Consequently, these techniques may be useful in predicting protein behavior. For example, loss of activity of therapeutic proteins can result from interfacial adsorption and conformational change and this may be influenced by different formulation additives. Interfacial rheology and tension techniques could be used to determine interfacial adsorption and interaction in the presence and absence of different additives and it may be possible to relate these changes to therapeutic activity. Interfacial rheology and tension measurement would also appear to be useful in preformulation studies on emulsifier films. The stronger the interfacial film, the more stable the resultant emulsion should be. The data provided by interfacial rheology and tension techniques are complementary and therefore it is suggested that these techniques be used together to obtain a more comprehensive knowledge of protein films. Interfacial tension is an indicator of adsorption and interfacial rheology is an indicator of adsorption and interaction.

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## REFERENCES

1. Burgess, D. J., Longo, L., and Yoon, J. K., *J. Parenteral Sci. Technol.* **45,** 239 (1991).
2. Burgess, D. J., Yoon, J. K., and Sahin, N. O., *J. Parenteral Sci. Technol.* **46,** 150 (1992).
3. Burgess, D. J., *in* ''Biotechnology and Pharmacy'' (J. M. Pezzuto, M. E. Johnson, and Manasse, H. R., Eds.), p. 116. Chapman and Hall, New York, 1993.
4. Manning, M. C., Patel, K., and Borchardt, R. T., *Pharm. Res.* **6,** 903 (1989).
5. Chmelik, J., Anzenbacher, P., and Kalous, V., *Czech. Chem. Commun.* **54,** 536 (1989).
6. Graham, D. E., and Phillips, M. C., *J. Colloid Interface Sci.* **70,** 403 (1979).
7. Graham, D. E., and Phillips, M. C., *J. Colloid Interface Sci.* **70,** 415 (1979).
8. Graham, D. E., and Phillips, M. C., *J. Colloid Interface Sci.* **70,** 427 (1979).
9. Graham, D. E., and Phillips, M. C., *J. Colloid Interface Sci.* **76,** 227 (1980).
10. James, L. K., and Sherman, P., *Biorheology* **13,** 79 (1976).
11. Moules, C. A., and Warburton, B., *in* ''Rheology of Food, Pharmaceuticals and Biological Materials with General Rheology'' (R. E. Carter, Ed.), p. 211. Elsevier, Amsterdam, 1990.
12. Dickinson, E., and Stainsby, G., *in* ''Advances in Food Emulsifiers and Foams,'' p. 142. Elsevier, London, (1988).
13. Peter, T., Jr., *in* ''The Plasma Proteins'' (F. W. Putnam, Ed.), p. 133. Academic Press, New York, 1975.
14. Hunter, M. J., and McDuffie, F. C., *J. Am. Chem. Soc.* **81,** 1400 (1959).
15. Behrens, P. Q., Spiekerman, A. M., and Brown, J. R., *Fed. Proc.* **34,** 591 (1975).
16. Beale, D., and Feinstein, A., *Q. Rev. Biophys.* **9,** 135 (1976).
17. Chothia, C., Lesk, A. M., Gherardi, E., Tomlinson, I. M., Walters, G., Marks, J. D., Llewelyn, M. B., and Winter, G., *J. Mol. Biol.* **227,** 799 (1992).
18. Sherriff, M., and Warburton, B., *Polymer* **15,** 253 (1974).
19. Yoon, J. K., and Burgess, D. J., *J. Colloid Interface Sci.* **151,** 402 (1992).
20. Morris, E. R., *in* ''Gums and Stabilizers for the Food Industry'' (G. O. Phillips, D. J. Wedlock, and P. A. Williams, Eds.), p. 3. Elsevier, London, 1986.