Proteins as Emulsifiers: Methods for Assessing the Role

CHARLES J. CANTE, General Foods Corporation, Kankakee, Illinois 60901;
ROGER W. FRANZEN and **FOUAD Z. SALEEB,** General Foods Corporation, Tarrytown, New York 10591.

ABSTRACT

The objective of this review is to present a variety of surface and colloid chemical methodologies, in brief, with which one may study the role of proteins as emulsifiers and co-emulsifiers. The techniques range from microtechniques aimed at single interfaces (film balance) and double interfaces (tensiolaminometry) to macrotechniques aimed at the gross emulsion (microcalorimetry, electrophoresis, pulsed, nuclear magnetic resonance, microwaves). Equipment, procedures, typical results, and interpretation of the data are reviewed. Some results with commercially available proteinaceous materials are presented. A link between emulsion formation and stability theory, experimental methods, and food applications is made through the examples of a salad dressing emulsion and a foamable emulsion.

INTRODUCTION

Foods are complex biosystems in which many constituent interactions can take place. Recent developments in food science have singled out protein as an important food constituent nutritionally as well as functionally. The components in food biosystems which can react with proteins include water, lipids, carbohydrates, minerals, vitamins, pigments, and others.

In food processing it is often necessary to blend edible fats and oils with various hydrophilic materials in an emulsion system. When considering emulsions, four classes of stabilizing agents can be distinguished: (a) inorganic electrolytes, (b) surface-active detergents, (c) finely divided insoluble solids, and (d) macromolecular emulsifying agents such as proteins, gums, and starches. The application of ordinary emulsifying agents is severely restricted because of food hygiene and food legislation requirements. Therefore, one of the most important functionalities of proteins is their ability to form (initiate) and stabilize oil/water emulsions in meat and/or nonmeat systems.

In using proteins as emulsifiers, it can be generally stated that the role of proteins is approximately parallel to that of nonprotein emulsifiers in nonprotein systems. In particular, the protein molecules are transferred from the bulk phase(s) to the oil/water interface. This protein adsorption lowers the interfacial tension and hence lessens the mechanical energy required to produce a given emulsion particule size (compared with no emulsifiers present). After emulsion formation the protein film at the interface serves an even more important role in preserving the stability of the emulsion by retarding the rate of coalescence of the oil droplets. It should be mentioned that prediction of protein emulsifying properties is much more difficult than with regular nonprotein emulsifiers. Proteins are complex polymeric molecules, and the functional properties for a given protein are greatly influenced by the "ionic" environment in which it is present (during an emulsification process). In particular, pH, ionic strength, nature and valency of the ionic species in solution (Ca, phosphate, etc.), the presence or absence of co-emulsifiers (ionic and nonionic surfactants) and other macromolecular stabilizers such as gums affect functionality.

Ionic and molecular species in solution through ionic as well as molecular association with proteins adversely modify the solubility, electric charge, molecular size and shape, rate of adsorption, and film properties of the proteins at the oil/water and air/water interfaces. Since various proteins have different chemical structures and molecular configurations, it is to be expected that the general rules governing emulsifiers will not be directly applicable to all proteins. However, in spite of this diversity and complexity, the protein molecules can be treated as colloidal macromolecules, and the general rules of colloidal chemistry are applicable in most cases. Some of the techniques that can be used to study interactions of proteins with their aqueous environment are discussed briefly.

In particular, surface and colloidal chemical methodologies needed to obtain information on protein-oil and protein-cosurfactant interactions to explain the protein's function are presented. Particular attention is given to film balance (single film and duplex film) and tensiolaminometric (double film) techniques for establishing the mechanism of action of the protein at the oil/water interface. Electrokinetic techniques are also employed to explain the effect of charged interfaces, pH, and ionic strength on the protein's emulsification character. Correlation of surface and colloidal chemical properties and performance in actual emulsions will be offered.

EMULSION FORMATION AND STABILITY

Formation

Emulsions are formed by dispersing one material into another where the materials are immiscible. The ease of formation is related to the work required to reduce the size of the dispersed phase, i.e., increasing the surface area. This work is described by equation I.

$$\Delta G = \int_{A_o}^{A_f} \gamma_i \, dA$$

Where dA is the change in surface area of the dispersed phase, A_o and A_f are the initial and final surface areas, and γ_i is the dynamic surface (interfacial) tension, not the equilibrium interfacial tension. Materials which can adsorb onto interfaces lower the interfacial tension and hence lessen the mechanical energy required to produce a given emulsion particle size. The lower the initial interfacial tension, the easier it will be to form the emulsion. What this means practically is that the rate at which a material transfers from the bulk phase and penetrates into an oil/water interface markedly affects the ease of formation of the emulsion whether or not that material stays in the interface to help stabilize the emulsion.

An example of this can be found in recent work by Rosano et al. (2), who found that the addition of short chain alcohols to one component of an emulsion prior to formation resulted in a transient lowering of the interfacial tension to practically zero; when the components were mixed, the emulsion formed quite readily. The transient lowering of γ_i was due to the rapid transport of the short chain alcohol from one phase of the emulsion through the interface into the next phase.

The techniques examined herein enable one to deter-

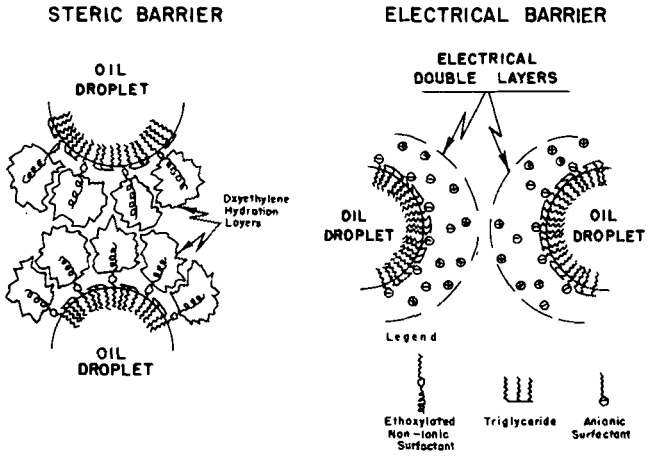

FIG. 1. Steric and electrical barriers in emulsion stability.

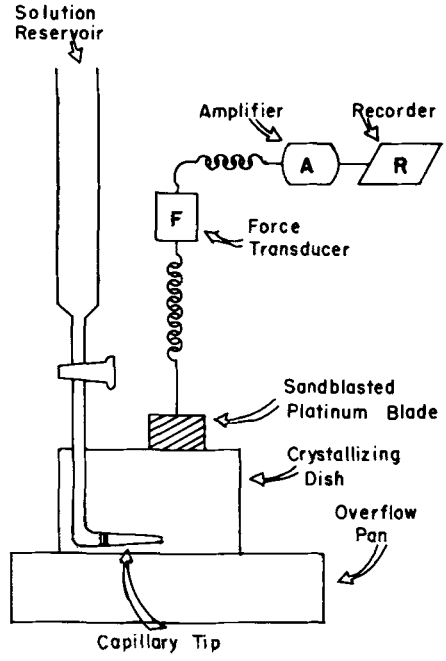

FIG. 2. Apparatus for determining dynamic surface tensions.

mine the dynamic as well as equilibrium interfacial tensions.

Stability

Emulsions usually destabilize either by (a) sedimentation (creaming up or creaming down) due to density differences between the dispersed phase droplets and the continuous phase, or (b) flocculation followed by coalescence (3). Barriers to destabilization are either steric and/or electrical in nature (Fig. 1).

It is generally accepted that at or near the isoelectric point proteins stabilize emulsions by a mechanism of adsorption and interfacial denaturation to produce a physical barrier (steric) against coalescence of dispersed droplets or particles. The techniques examined herein enable one to assess filmforming properties of proteins in combination with lipids and surfactants. In particular, one desires to know the type and strength of the film, its compressibility, and its charge.

METHODOLOGIES

Rate of Adsorption

The rate of adsorption can be determined from dynamic surface tension measurements at the air/solution interface made by the Wilhelmy wettable plate method (4). The apparatus (Fig. 2) consists of a suspended sand blasted, platinum blade from a force-measuring transducer to measure the surface tension in a crystallizing dish filled to the rim with the solution to be studied. (The dish can be set in an overflow container.) A 250 ml separatory funnel with a long glass stem, which has a right angle bend at the bottom end, is also filled with the test solution. The right angle bend of the funnel is placed into the solution flush with the bottom of the crystallizing dish. The solution is allowed to overflow into the dish, thereby creating a new surface. The basic experiment is to overflow with 10 ml of test solution and record the surface tension with time until equilibrium is reached. These data can be plotted as surface tension vs. the square root of time to obtain a straight line (See Ref. 4, Fig. 1).

Electrokinetic Measurements

Proteins are colloidal particles and have an electric charge of a certain sign and magnitude at a given pH. Each pure protein is characterized by having an isoelectric point (iep). At that pH the number of the positive charges are equivalent to the number of negative charges. For many proteins the solubility is least at the iep due to the loss of the contribution of the electrical repulsion forces of the particles. However, in food systems, the iep of proteins loses its meaning. In such complex systems pseudo-isoelectric points can be achieved at different pHs by interactions of anionic surfactants with positively charged proteins and vice versa and/or in the presence of multivalent ions (e.g., Ca^{++} and PO_4^{---}).

The sign and magnitude of the charge on protein particles dispersed in water is determined indirectly from the zeta potential (ξ), using one or more of the electrokinetic phenomena (1) e.g., electrophoresis, electro-osmosis, streaming potential, sedimentation potential, and electroviscous effects.

The electrokinetic or zeta potential (ξ) of proteins is conveniently calculated (1) from the electrophoretic velocity (ν) of the particles (using for example a Zeta-meter), via the Smoluchowski equation:

$$\xi = (4 \Pi \eta \nu)/DE$$

where Π=3.14, D and η are the dielectric constant and viscosity of the dispersing medium respectively, and E is the applied electric field strength. It is evident from equation II that ξ is independent of particle shape.

Film Balance Methods

Single film techniques: A typical experimental apparatus (Fig. 3) for measuring surface isotherms has been described by Christodoulou and Rosano (5), and Rosano, et al. (6). The temperature of the substrate can be regulated by circulating water from a constant temperature bath through a glass cooling coil submerged in the substrate. The pH and ionic strength of the substrate may be controlled as desired. An automatic barrier drive with variable speed control permits determination of an optimum compression rate and reproducible π-A and $\Delta\nu$-A surface isotherms.

Surface pressures (π) are determined from surface tension measurements, which are made by suspending a sand blasted platinum blade from a microforce transducer-amplifier where the output is recorded continuously. Surface pressure, π, is defined as $(\gamma_o - \gamma_i)$ where γ_o and γ_i are the surface tensions of the clean and film-covered surfaces, respectively.

Surface potentials ($\Delta\nu$) are measured with an air-ionizing electrode (5,6) (radium-226 source) placed 1 to 2 mm above the surface of the liquid surbstrate and connected to a precision potentiometer, a high input resistance electrometer, and a reference electrode (Ag/AgCl or calomel) dip-

ped into the bulk of the aqueous substrate. The e.m.f. of the cell composed of the radioactive electrode, reference electrode, potentiometer, and electrometer, all connected in series, is measured immediately after cleaning the surface of the aqueous substrate (ν_o), and compared with the e.m.f. obtained after spreading a film on the surface (ν). The difference between the two e.m.f.s (ν-ν_o) is $\Delta\nu$, the surface potential.

$\Delta\nu$measurements yield information about the polarity of the interface since the interfacial region is qualitatively described (1) as a parallel plate condenser with a plate separation X and a charge density δ. Then

$$\Delta\nu = (4\Pi \times \delta/D)$$

where D is the dielectric constant. Furthermore the charge density is assumed to arise from the effective dipole moment, μ_o, of the oriented interfacial film. Hence

$$\Delta\nu = (4\Pi N\mu_o/D)$$

where N is the number of dipoles per square centimeter. Then $x\delta = Nex = N\mu_o$, e is the electron charge and $\mu_o = \mu_a \text{Cos}\theta$ i.e., μ_a is the actual dipole moment, and θ is the angle of inclination of the actual dipoles to the normal (1).

From the surface isotherms, one may also determine the compressibility, K, of the film (1):

$$K = -(1/A)\,(\partial A/\partial\pi)_T$$

In a typical experiment, a fat or oil monolayer is deposited on the aqueous surface from a solution in n-hexane with a micrometer syringe. The (π-A) and ($\Delta\nu$-A) characteristics of the fat or oil are determined at a given temperature and for a given aqueous phase. Then, one of several experiments may be conducted.

The first is ($\partial\pi/\partial t$) and ($\partial\Delta\nu/\partial t$) at constant area (A) in which one sets the monolayer to a fixed area/molecule and then injects either a protein solution or a surfactant solution into the aqueous phase, and the increase of π vs. t is recorded. This enables one to determine the ability of a protein or surfactant to adsorb onto or penetrate into a spread oil or fat film vs. the initial state of that film. Once equilibrium is reached, one repeats the π-A and $\Delta\nu$-A isotherms and compares these to the initial state. From this, one can determine whether the protein has penetrated the film or adsorbed onto it; and if it has penetrated the film, one can assess the strength of the protein-fat or protein-oil interaction (Fig. 4). Then, one can re-expand the film to a given area/molecule and inject a surfactant solution into the aqueous phase and determine how the particular surfactant interacts with the protein-fat or protein-oil film. The sequence can be reversed; i.e., fat (oil) → surfactant → protein to determine the effect of order of addition on the kinetics, type and strength of interaction. The following types of questions can be answered:

– Does the protein interact with the fat (oil)?
– Does the order of addition impact the final film?
– Does the surfactant strengthen the film, or does it cause the protein to desorb from the film?

For a given fat (oil), one can determine the effect of protein-type, concentration, pH, ionic strength, salt-type, surfactant-type (ionic, nonionic, oil soluble, watersoluble) on interfacial film properties.

The second possibility is to determine $\left(\frac{\partial A}{\partial t}\right)$ and $\left(\frac{\partial \Delta V}{\partial t}\right)$ at constant surface pressure (π). In this case, the area/molecule expands to maintain the surface pressure, as the protein and surfactant interact with the film. The reader is referred to some of the excellent published studies (7-15) for specifics on theory and applications.

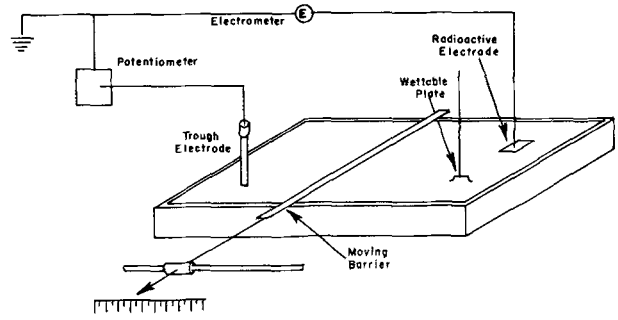

FIG. 3. Schematic Diagram of Film Balance.

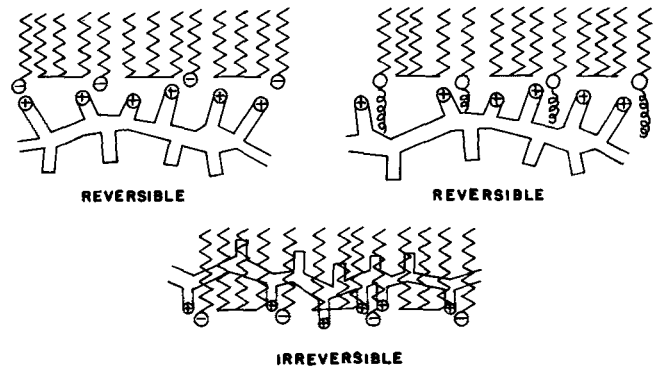

FIG. 4. Types of protein-surfactant interactions.

In Table I the results for the penetration of triglyceride films (oil and fat, respectively) by sodium caseinate, a milk whey fraction and a surfactant sorbitan monostearate polyoxyethylene (20) [SMSPOE (20)] are given (16). One would interpret these results as follows: (a) for an emulsion containing fat, either the surfactant or the milk whey fraction alone or the surfactant-sodium caseinate or surfactant-milk whey fraction combinations would suffice as emulsifier systems, but sodium caseinate alone would not; (b) for an emulsion containing oil, either the milk whey fraction alone or the milk whey fraction-surfactant combination would suffice as emulsifier systems while the other materials would not. A good emulsifier system gives strong film penetration, condenses the film and may raise the collapse pressure. A poor emulsifier system either weakly penetrates or does not penetrate at all, expands the film and may lower the collapse pressure.

Duplex film technique: In the single film technique, one assumes that information generated with a monolayer of fat or oil will relate to behavior observed with an oil droplet because the physicochemical action of formation and stability occurs at the interface. One can embellish upon the single film technique by spreading a thicker oil layer instead of a monolayer and conducting the experiments at the interface. This technique is tedious but can be rewarding (17).

Tensiolaminometry

Tensiolaminometry is the outgrowth of the pioneering experiments of Thibaud and Lemonde (18) two decades ago, in which Matalon (19-22) designed an apparatus which provided a measure of both the ease of foam formation and its subsequent stability by examining both the ease of formation of thin liquid lamellae generated within a vertical frame from a solution and the lamellar stability. Eydt and Rosano (23) and Cante and Moreno (16) modified the instrument and used it to study oil/water interfaces and to screen for emulsifiers and foaming agents.

The apparatus consists of a wettable platinum wire frame inside which a liquid lamella is formed when the frame is withdrawn from a solution. With our instrument,

TABLE I

Penetration of Triglyceride Films and Complex Formation[a]

Penetrant	Film Penetrated	Steady State Penetrating Pressure, πp (Dynes/CM)	π-A character
SMSPOE(20)	Fat	13.5 ± 1.0	+C, $+\pi_c$
NaCaseinate	Fat	13.0 ± 1.0	E, $-\pi_c$
M.W.F.	Fat	14.5 ± 0.5	LC
NaCaseinate	Fat-SMSPOE(20)	10.0 ± 1.0	+C(Fat-NaCas.) +E(Fat-SMSPOE(20))
M.W.F.	Fat-SMSPOE(20)	11.0 ± 1.0	LC to C
SMSPOE(20)	Oil	5.5 ± 2.0	+E
NaCaseinate	Oil	4.5 ± 1.0	E, $-\pi_c$
M.W.F.	Oil	12.5 ± 0.5	LC to C
NaCaseinate	Oil-SMSPOE(20)	7.0 ± 1.0	E
M.W.F.	Oil-SMSPOE(20)	11.0 ± 1.0	LC to C

[a]C = condensed, LC = liquid condensed, E = expanded, + = more or higher, - = less or lower, π_c is the film collapse pressure, and the parenthetical item is the comparison base. The absence of a parenthetical item means that the comparison base is the fat or the oil as appropriate.

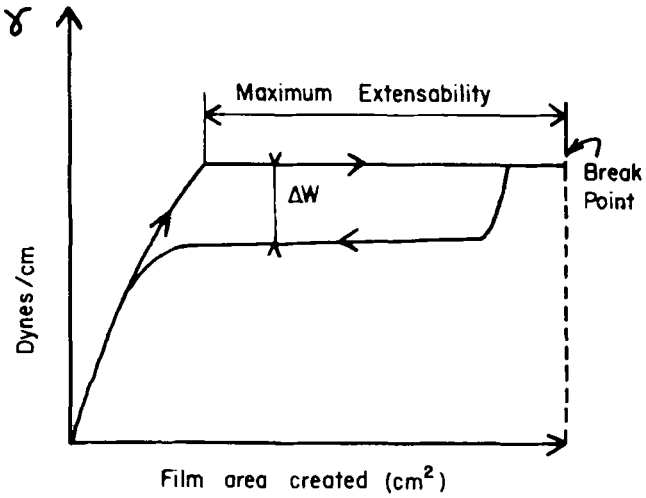

FIG. 5. Typical tensiolaminometry results.

the frame is fixed, and the solution can be lowered or raised by means of a movable table actuated by a variable speed motor-gear system combination. The force acting upon the frame is continuously measured with a micro-force transducer and recorded. With a frame width of 2.0 cm, the surface tension can be measured within ±0.25 dyne/cm. The rate of film formation may be varied from 0.06 to 0.60 cm^2/sec.

The tensiolaminometer had not been widely used until the late 1960s because the force had to be measured with a horizontal torsion balance wherein the torsion of the wire was determined optically by photographing the deflection of a luminous spot. This method was tedious and cumbersome. The development of the force transducer-recorder combination eliminated this instrumental difficulty inherent in an optical system.

To study emulsification properties, one prepares a system with an oil layer over an aqueous layer. The appropriate layer contains the material to be studied; i.e., protein, surfactant or combination thereof. The frame is submersed in either layer, and the experiment involves moving the frame into the next layer. Hence, oil-in-water or water-in-oil films can be studied. Figure 5 demonstrates a typical result one obtains with this instrument. One can readily study the effect of pH, ionic strength and temperature on emulsion stability with this technique simply by altering pH and ionic strength of the phases and thermostating the systems.

As the nature of the film around the air bubbles largely determines the stability of the foam, so the nature of the adsorbed film at the oil/water interface determines emulsion stability. Eydt and Rosano (23) found that with emulsifiers having a low hydrophobic-lyophilic balance (HLB value), oil-in-water films are obtained which are irreversible. These emulsifiers promote water-in-oil emulsions. A strong oil-in-water film suggests good stability, as such a film has to be broken for two water droplets to coalesce. When the HLB of the emulsifier is increased to 7, oil-in-water films are still obtained, but they are now completely reversible. The emulsion corresponding to that HLB value is of poor stability. Using this technique, Eydt and Rosano (23) and Cante and Moreno (16) have studied systems containing either sodium caseinate, bovine serum albumin or proteose peptone in the aqueous phase either alone or in combination with various TWEENS and SPANS or other food grade surfactants and edible grade oil phases, such as olive oil and safflower oil. Similar results are obtained regarding emulsion stability when one compares tensiolaminometric data with gross emulsion stability.

Microcalorimetry

Proteins in their natural environment are known to bind varying amounts of different fatty acids (24). Protein isolates also interact with different ionic surfactants (25) with subsequent and sometimes dramatic modifications of their functional properties. Titration microcalorimetry is a newly employed technique that provides information on protein binding sites and the binding energies involved. The surfactant solution is added automatically and continuously into the reaction vessel containing the protein solution. The amount of heat (millicalories) evolved or adsorbed for the specified time interval is then plotted against the total surfactant concentration after corrections are made for chemical (dilution) and nonchemical effect (baseline drift).

Saleeb and Schnez (26) found that ionic surfactant binding by proteins is generally characterized by two exothermic peaks (Fig. 6). The first peak at low surfactant/protein ratios is invariant at pHs above and below the isoelectric point of the protein indicating binding that is predominantly hydrophobic in nature. However, for a given protein the height of this first peak is higher at pHs above rather than below the iep. This fact may be explained by the increased number of exposed hydrophobic sites on the protein at pHs above the iep (24). The binding at higher surfactant/protein ratios (second peak of the thermometric titration curves) is dependent on pH and points to some type of protein/surfactant binding initiated by ionic interactions. The magnitude of the second peak for different protein-surfactant systems coupled with foam studies, zeta potential and emulsion stability measurements indicates that in this high surfactant region, surfactant ions interact ionically with oppositely charged ions on the protein surface (26). This type of interaction is followed by a second layer of surfactant molecules adsorbed in a tail to tail manner. The above results were also confirmed by monolayer studies of

protein-surfactant mixtures by Schenz and Saleeb (27).

Emulsification Capacity

One of the most widely used methods for studying the ability of a protein to emulsify oil (fat) is termed Emulsification Capacity (EC). The method was first developed by Swift et al. (28, 29) to study the factors that influence meat emulsions. It is used in model systems in an attempt to quantify and/or eliminate many of the uncontrolled variables encountered under larger scale commercial conditions. EC is defined as the volume of oil emulsified by a given unit of sample at the point of phase inversion; i.e., o/w emulsion inverting to w/o emulsion. It is usually expressed as milliliters oil emulsified per gram sample, or based on the quantity of protein or nitrogen present per unit sample.

In essence, the methods consists of adding a protein solution with or without other additives or meat extract solution to a jar. A quantity of oil or melted fat to be studied is added, and a high speed cutting-mixing (ca. 13,000 rpm) is initiated.

Additional oil or fat is then immediately added at a constant rate. An o/w emulsion is formed which gradually increases, then sharply decreases in viscosity. At this point, oil/fat addition is terminated, and the total volume of oil or fat present in the collapsed emulsion is recorded. The point of maximum viscosity indicates the maximum quantity of oil or fat which can be held in an emulsion by the sample. Once this quantity is exceeded, the emulsion breaks, and the viscosity falls.

Webb et al. (30) eliminated the subjectivity of visually observing emulsion collapse (inversion) while determining EC through the measurement of electrical resistance during formation and collapse of dilute emulsions. This provided an objective and continuous record for end-point determination. Crenwelge et al. (31) utilized the increased amperage requirements of a Waring blender due to increasing viscosity followed by a sharp drop in amperage at inversion to detect the end point in (EC) of more concentrated emulsions. Marshall et al. (32) advocated use of an oil soluble stain to increase the precision of visual end-point determination of either high or low viscosity emulsions.

Saffle (33) described many of the variables which affect the EC determination of proteins involved in meat emulsions. Such factors as equipment design, container shape, rpm of blending, rate of oil addition, temperature, pH, protein source (concentration and solubility), type of oil, salt type and concentration, sugars and water content contribute to variability observed among different laboratories.

Table II presents emulsification capacities determined by Balmaceda et al. (34). One can see from the data that proteins with high solubility have high emulsification capacity. Several researchers, e.g., Saffle (33), Pearson et al. (35), Yasumatsu et al. (36), and Crenwelge (31), also noted a reasonable correlation between emulsification capacity and protein solubility (and factors affecting solubility, e.g., pH, ionic strength, salt type, and temperature). Also, correlation between emulsification capacity and protein surface area has been noted by Saffle (33).

In Saffle's study of meat emulsions (33), he generalized that the salt soluble proteins are much more efficient emulsifiers than the water soluble proteins. He explained these differences as being due to protein shape and surface area, approximating these parameters from viscosity data. The salt soluble proteins were found to have ca. 50 times as much surface area to surround fat particles as compared to the water-soluble proteins.

Pearson et al. (35) evaluated the EC and stability of soy, sodium proteinate, potassium caseinate, and nonfat dry milk, (all of which may be used as protein additives in sausage products) and concluded that protein solubility is closely related to emulsifying capacity and that pH probably influences EC in an indirect manner by affecting the solubility of the proteins. This work, as well as the work of Borchert et al. (37) on the electron microscopy of meat emulsions, shows microscopic evidence that fat is encapsulated by protein membranes or layers.

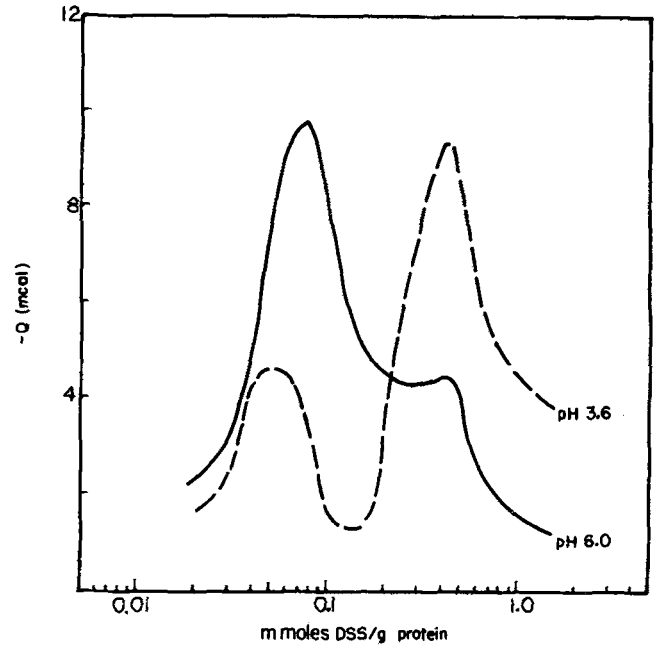

FIG. 6. Microcalorimetry of sodium caseinate with sodium dioctyl sulfosuccinate (DSS).

TABLE II

Emulsion Capacity of Various Protein Materials

	EC[a]	Solubility (pH 7)[b]
Egg white (P110)	100	95.4
Caseinate products	40-100	65-100
Solac 500 (lactalbumin)	79.5	96.1
Pea flour	7.3	
Peanut flour	9.7	
Oat flour	11.9	
Soy flour	12.0	
Sesame flour	9.8	
Wheat gluten (ADM)	13.9	7.0
CP 90 (corn isolate, CPC)	13.2	11.8
Yeast (Amoco E51P)	16.4	1.8
SCP (Campbell's)	14.3	10.1
Fish (Astra-Nutrition)	10.8	18.6
Fish (USDC, succinylated)	41.3	100.0

[a]EC = ml oil/100 mg protein.
[b]Solubility = % Soluble of 0.1% protein in water (filtration method).

Yasumatsu et al. (36) evaluated the emulsifying properties of various soybean protein products in more complex model systems which simulated actual food compositions. Emulsifying properties were found to correlate positively with soluble protein and negatively with fiber contents.

Smith et al. (38) evaluated the EC and emulsion stability of eleven protein additives in model systems, as well as in high and low fat frankfurter emulsions (3.5% level). The additives included soy protein isolates, concentrates and flours, cottonseed flours, all with high and low nitrogen solubilities (HNS, LNS), nonfat dry milk (HNS), and fish protein concentrate (LNS). They concluded that in frankfurters containing low fat (ca. 25%), the protein additives exerted little influence on stability, while in those containing high fat (ca. 35%) the additives exerted a significant influence on emulsion stability. A hypothesis based on microscopic observation was discussed which suggested that very small particles (finely divided solids) can aid emulsion

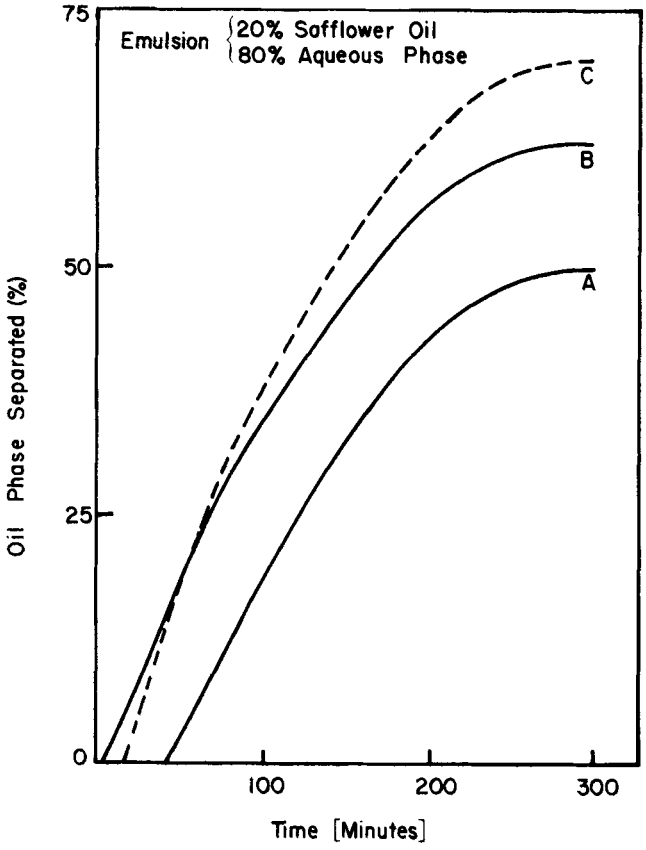

FIG. 7. Emulsion stability from volume of oil expelled vs. time. A = No HCl; B = 1 ml.; C = 1.4 ml. of 0.1N HCl.

stabilization based on physical state vs. solubility. Alternately, they state that solids preventing coalescence of fat droplets may be a factor in explaining the effect of good emulsion stabilities with fish and soy protein concentrates of LNS. This hypothesis is worthy of consideration, since both concentrates exhibited low EC in dilute systems.

Crenwelge et al. (31) compared the EC of glandless cottonseed flour (57.5% protein), soy concentrate (67.5%), decolorized bovine hemoglobin (90.1%), and low-heat nonfat dry milk (35.4%) and determined the optimum pH (highest solubility) at which to compare the EC of the protein samples. These pHs were (soy) 9.4, (globin) 3.1, (cottonseed) 8.9, and pH 7.1 for milk protein. The trends indicated good correlation between protein solubility and EC. Through pH optimization lower protein concentrations were required to reach maximum EC compared to pHs near neutrality. When all the variables (blender speed, pH, etc.) were optimized for each protein, bovine globin had the greatest EC. Furthermore, Crenwelge et al. (31) concluded that although globin produced the best emulsions on a unit protein basis, soy and cottonseed in particular may vary in emulsifying properties with the method of isolation. This statement is critical, since conditions of isolation or processing which promote protein denaturation and reduce solubility (e.g., certain complexing agents, extremes of pH or temperature, etc) will be reflected in alteration of the emulsifying properties of the protein sample.

Emulsion Stability

Thermodynamically all emulsions are unstable and break to different extents with time. The rate of globule coalescence is greatly dependent, among other things, on droplet size, number of droplets per unit volume and the thickness and the nature of the emulsified/stabilizer layer at the oil/water interface.

It is found that the time required for emulsions to break may vary from a few seconds to several years. Since most work on emulsion stability involves changes in the original emulsion structure, such as dilution (as in counting), mechanical forces (as in centrifugation) or temperature variations, these techniques only give relative stabilities under these specified conditions. This is the major reason that in most cases agreement between different investigations is lacking. In spite of these limitations, it is always necessary to know the stability of an emulsion. Stability of emulsions can be determined by several techniques. Some of these are summarized next.

1) *Extent of creaming or coalescence by measuring the volume of the different phases.* Construction of fat volume vs. time curves are helpful in overcoming the difficulty of observing the phase boundary at the initial stages of separation. Recently Saleeb et al. (39) used this technique to characterize oil/water emulsions stabilized with fatty acids at different pH values (Fig. 7).

In systems with higher stabilities, an accelerated destabilization of the emulsion is achieved for ease of measurement. For example, Trautman (40) and Vold (41) used centrifugation to determine the kinetics of demulsification of a number of systems. Yet in another method, freeze-thaw cycles or simple heating were used to accelerate the rate of emulsion breakdown. When gelling is not operative, higher temperatures in most cases enhance coalescence (3) due to the increased kinetic energy of the particles and hence a higher probability of demulsification as described by Smoluchowski's equation:

$$n = n_o / (1 + an_o t_i)$$

where $a \approx (kT/6\Pi\eta)$ and n_o and n are the number of droplets at t=o and $t=t_i$, respectively. The use of freeze-thaw cycles is particularly valuable for systems in which the structure of the interfacial adsorbed layer is disrupted by freszing. However, quite recently Raymond (42) reported that in the presence of fish glycoproteins, freeze-thaw cycles enhanced emulsification of mineral oil.

2) *Measurement of the number and size of droplets as a function of time.* Direct counting of dilute emulsions is generally achieved using a Coulter Counter of photomicrographs while reflectance measurement is an indirect method to follow emulsion stability that depends on the change in number and size of droplets as function of time. These two techniques are generally used to test the theory of stability. Using this technique, Mita et al. (43) found that the energy barrier for coalescence of benzene globules stabilized with bovine serum albumin to reach an optimum (2.33 Kcal/mole) at the isoelectric point of the protein. This finding is in agreement with the general rule that proteins show a maximum in foamability and adsorption at their iep, provided they are soluble in water at that pH.

3) *Pulsed NMR of dilute or concentrated oil/water emulsions is a very useful tool in scanning the effect of time on oil distribution at different levels of an emulsion in a graduated cylinder.* The method is being used in GF labs to determine the stability of vegetable oil emulsions stabilized by different proteins (44).

4) *Microwave irradiation creates heat by passing through a body.* The amount of heat created is proportional to the dielectric constant of the medium. Since the dielectric constant of water is much higher than that of oil, it is expected that the surface temperature of the more stable emulsions (less oil rich) should be higher than that of the less stable emulsions (45). Also, the temperature difference between the surface and the bottom should be less for the more stable emulsion than for the less stable emulsion. In this respect, the microwave method produces information on the stability of emulsions in their natural environment. The same is true with pulsed NMR, which is a much simpler technique.

APPLICATIONS TO FOOD SYSTEMS

Let us examine how to apply the foregoing techniques to food systems by considering the case of two emulsions – one is to be stable as such (salad dressing), and the other is to be foamed (dessert topping). Furthermore, let's assume that both emulsions contain similar ingredients, namely water, a triglyceride, chemical emulsifiers (ionic and nonionic), a soluble proteinaceous ingredient, a gum and salts.

Clearly one would like to know the effect of pH and salt concentration on the magnitude and sign of the charge for the proteinaceous ingredient, the gum and chemical emulsifier and the extent of triglyceride-chemical emulsifier film penetration by the protein and gum. From this information one can then identify conditions for maximum and minimum polar-polar interactions and hydrophobic interaction. This information can be derived from zeta potential measurements and π-A and $\Delta\nu$-A isotherms.

In the case of the salad dressing emulsion, one wants to maximize the interaction between the protein and chemical emulsifiers to provide a strong film with which to stabilize the emulsion droplet, but one does not want the protein to complex with the gum, thereby reducing the surface activity of the protein (stability) and the hydrodynamic property of the gum (viscosity building). Furthermore, the film must have reasonable compressibility with which to resist permanent damage from and recover from thermally or mechanically induced deformations due to temperature gradients or collision with other droplets, respectively. One chooses the gum, pH, ionic strength and salt type to optimize emulsion stability.

On the other hand, in the case of the foamable emulsion, one wants to minimize the interaction between the protein and chemical emulsifiers since both will be needed to produce and stabilize the foam bubbles. Still one wants to prevent complex formation between the gum and the protein since each has a role in foam stability. One can assess the foamability and foam stability of the emulsion by tensiolaminometry.

The film balance, tensiolaminometric and electrokinetic techniques discussed herein allow one to generate voluminous data with a minimum quantity of experimental materials. The findings can be evaluated and tested with microscopic techniques.

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