# Preparation and Surface Activity of Modified Soy Protein

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**ABSTRACT:** A series of novel surfactants have been prepared by the reaction of hydrolyzed soy protein with alkyl succinic anhydride. These novel surfactants exhibit excellent surface active properties including surface tension, foaming, emulsifying, wetting power, and buffer ability. The hydrophobic modified protein exhibit more surface activity than original protein because of the enhanced hydrophobicity in structure. The increase in hydrophobic chain length leads to an increase in the surface activity. The surface tension reduction is correlated to the hydrophobicity of the modified molecule, which was determined by a fluorescent probe. In application on cotton bleaching procedures, these surfactants increase the whiteness of fabrics. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 102: 3498–3503, 2006

**Key words:** modification; synthesis; protein; surfactants; fluorescence

## INTRODUCTION

Since proteins are composed of both hydrophobic and hydrophilic amino acid, most of them have an amphipathic nature.[1] It is also known that the surface activity of protein increased apparently by the modification of structure to enhance its hydrophobicity.[2–5] Increased hydrophobicity of proteins can be achieved either by changing the structure or the molecular weight, or by covalent attachment of various hydrophobic groups to the protein molecule. The most common covalent attachments are acylation reactions, in particular on the lysine groups.

Various modified proteins that are prepared by the acylation reaction of proteins with the anhydride, acryl chloride, or *N*-hydroxy succinimide of carboxylic acid were reported by several investigators previously.[6–8] Some of products have been used alone as a polymer-like surfactants or incorporated in surfactants to improve the compatability of surfactants with skin.[9–11] However, the disadvantages have in terms of synthesis and practical application, including the unsatisfactory detergency and the undesirable impurity of high boiling solvents (used in synthesis process) in the end products. To avoid these disadvantages, a simple method has been performed in aliquots to prepare the gelatin derivative surfactants.[12,13]

In this article, we present the preparation and surface active properties of a series of modified soy protein surfactants. Surfactants are prepared by the reaction of hydrolyzed soy protein with alkyl succinic anhydride. The surface active properties of modified protein including surface tension, foaming, emulsifying, wetting power, buffer ability, and fluorescence properties are studied. The application on cotton fabric bleaching procedures is also studied

## EXPERIMENTAL

### Materials

Soy protein, (2-Dodecen-1-yl) succinic anhydride, and (2-Nonen-1-yl) succinic anhydride were obtained from Sigma, and methyl *tert*-butylether (MTBE) was obtained from ACROS. Reagent grade succinic anhydride, sodium hydroxide, and hydrochloride acid were purchased from Hayashi Pure Chemical and used without further purification. The fluorescence probe used was rhodamine B and it was supplied by KOCH-LIGHT Chemical.

### Synthesis of soy protein surfactants

Figure 1 illustrates that the soy protein surfactants were prepared from soy protein and alkenylsuccinic acid anhydride. A reaction mixture containing 20 g of soy was taken up in 80 g of water and 4 g (0.04 mol) of alkenylsuccinic acid anhydride were added with stirring at 70°C. The pH value of the reaction mixture was kept at 10 by addition of dilute sodium hydroxide. After a reaction time of 5 h, the mixture was cooled to room temperature and adjusted with dilute hydrochloride acid to a pH value of 7.

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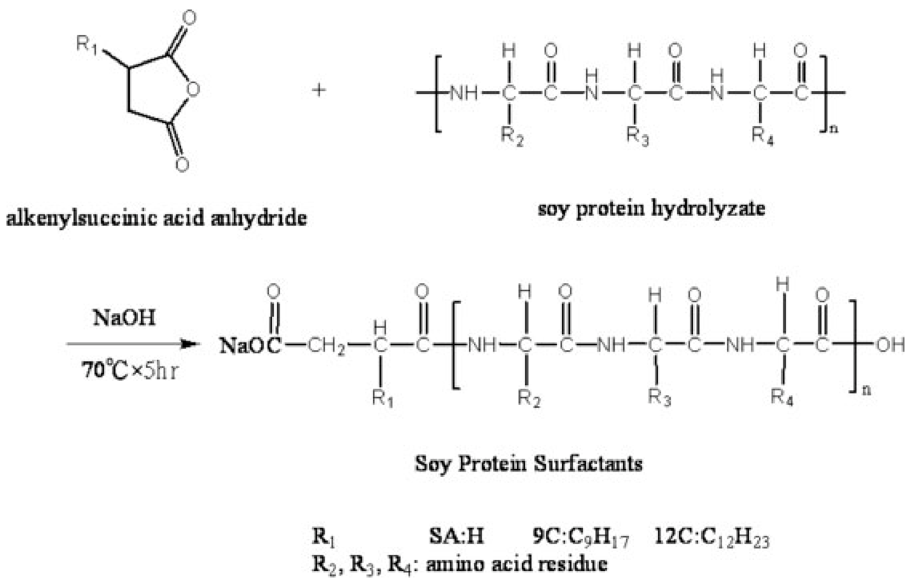


**Figure 1** Preparation of soy protein surfactants.

A water-insoluble products (mainly unreacted alkenylsuccinic acid) were removed from the reaction mixture by extraction with methyl *tert*-butylether (MTBE). Traces of MTBE were removed from the aqueous phase by heating to 40°C in a water jet vacuum.

### Analysis

The structure of the final products was confirmed by infra-red spectrum (IR) and proton nuclear magnetic resonance $^{1}$H-spectrum (NMR). IR spectra were obtained with a Japan Spectroscopic FTIR-3 Spectrophotometer, the sample was coated as a thin liquid film on thallium bromide/thallium iodide crystal surface. The thallium bromide/thallium iodide crystal is water insensitive. Nuclear magnetic resonance ($^{1}$H NMR) spectra were obtained with a Varian 360 L NMR, and the solvent used was $D_2O$.

### Measurements

Surface tension was determined at room temperature with a Japan Kaimenkaguka CBVP-A3 surface tensiometer. A 1.5% (by weight) surfactant solution was freshly prepared as stock solution and then diluted to the desired concentration for each measurement. Surface tension was measured three times for each concentration, and an average error less than 0.5 dyn/cm can be obtained routinely.

Contact angle, which describes the angle formed between the planes tangent to the surfaces of the solid and the liquid at the wetting perimeter, was measured with a FACE CA-5 contact angle meter. An acrylic plastic sheet and unscoured cotton fabric were used. Foaming properties were determined by the Ross-Miles method. Foam production was measured as the height of the foam initially produced, and foam stability was measured as the height after 3 min. Buffer ability was determined at room temperature with a solar pH meter.

Oil (10% w/w) in water emulsions were prepared by addition of 10 g of soybean oil to 90 g of 10 mg/mL soy protein surfactants solutions and homogenizing with IKA Labortechnik Ultra-Turrax T25 homogenizer, at 11,000 rpm for 5 min. The average diameter (by volume) and size distribution of the emulsion droplets was measured by a Microtrac S3000.

The emission spectrum of the solutions was measured by a Aminco-Bowman Series 2 Luminescence Spectrometer. The excitation wavelength was 350 nm, and the emission was measured between 370 and 800 nm. Hydrophobicity was evaluated by using the emission spectra of rhodamine B in $6 \times 10^{-5}M$ aqueous solutions. The probe solutions were prepared by dissolving the rhodamine B in deionized water and adding 20 g/L soy protein surfactant to the solution.

A rapid laboratory dyeing machine was used to study bleaching of a gray cotton fabric by hydrogen peroxide. The bleaching recipe included the following: $H_2O_2$ (20%), 10 g/L; NaOH, 5 g/L; $NaSiO_3$, 5 g/L; auxiliaries, 2 g/L; liquor ratio, 30 : 1; temperature, 80°C; and time, 40 min. After the bleaching, the whiteness of the cotton fabrics was measured using an Applied Color System (ACS, Color Matching System, CS-5) spectrophotometer.

## RESULTS AND DISCUSSION

### Preparation

A typical IR spectrum of the synthesized soy protein surfactants (Soy-12C), as shown in Figure 2, displayed bands at 3200–3550 cm$^{-1}$ (—OH, stretching),

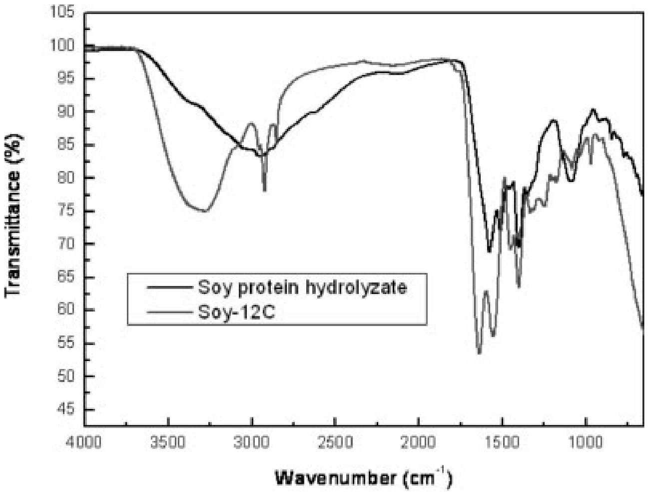


**Figure 2** FTIR spectra of soy protein hydrolyzate and Soy-12C protein surfactant.

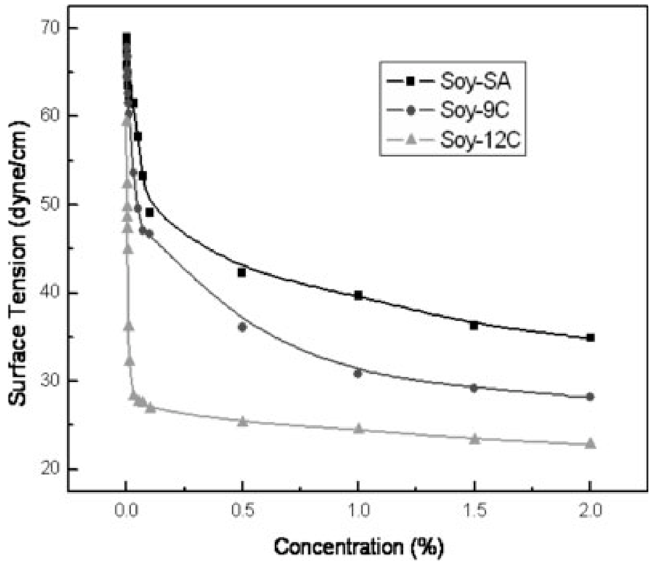


**Figure 3** Plots of surface tension against concentration of soy protein surfactants.

3100–3500 $cm^{-1}$ (N—H, stretching), 2926 $cm^{-1}$ (—$CH_2$, asymmetric), 2853 $cm^{-1}$ (—$CH_2$, symmetric), 1640–1700 $cm^{-1}$ (C=O, stretching), 1515–1550 $cm^{-1}$ (N—H, bending), and 1425 $cm^{-1}$ (C—N, stretching). The structure of unmodified soy protein was only NH group not OH, the intensities of NH stretching vibration at 3100–3500 $cm^{-1}$. The OH stretching peak intensity in the modified soy protein showed increase and shifted to 3200–3550 $cm^{-1}$ and meanwhile its shape became broad. The structure of the compounds was further supported by the $^1$H NMR spectrum, it gave signals at δ 0.9 ppm (—$CH_3$), 1.3 ppm (—$CH_2$), 1.4 ppm (—CH), 1.5–1.6 ppm (*R*—NH), 2–3.8 ppm (*R*—OH), and 6.8–7.4 ppm (—CONH).

## Surface tension

The surface activity depends on the chemical structure of the molecule. The important factor is the length of the hydrophobic fragment, but type and number of hydrophilic groups play a role as well. The surface tension reduction of aqueous solutions by the soy protein surfactant was evaluated at various concentrations. As shown in Figure 3, increased hydrophobic chain length improves the ability of the soy protein surfactant to reduce surface tension (i.e., the hydrophobic structure causes surfactant molecules to concentrate at the surface and reduces surface tension). The equilibrium surface tension is achieved more quickly by the longer chains at high soy protein surfactant concentration. These results may be attributed to the fact that an increase of hydrophobic portion of the soy protein surfactant results in an increase in its concentration at the surface.

## Wetting power

Addition of surfactants to water is a well-established means of enhancing the ability of aqueous solutions to wet and spread over solid surfaces. Textile materials are in frequent contact with surfactant solutions during their manufacturing or finishing process as well as during cleaning. Wetting behavior of fibrous materials determines their liquid transport and absorbent characteristics and is crucial to their manufacturing processes, functional properties, and end-use performances. Textile materials need to have good wetting properties to deliver chemicals uniformly to the fibers or between the fibers.[14,15]

Table I shows the contact angle formed between the aqueous solution of the soy protein surfactant and the surface of an acrylic plastic sheet or cotton fabric. These angles were smaller than those found with water, indicating that solutions of all the products possessed the power to wet acrylic plastic and the fabric. In addition, an increase in the hydrocarbon chain length of hydrophobic segment clearly decreased the contact angle, that is, Soy-12C possessed the minimum value contact angle, indicating that it is a better wetting agent than other products.

## Emulsifying power

The quality of an emulsion depends on the droplet size of the dispersed phase. Usually, the aim of emulsifica-

**TABLE I**
**Contact Angles of Soy Protein Surfactants**

| | Contact angle (degree) | |
|---|---|---|
| Compound | Acrylic plastic | Cotton fabric |
| $H_2O$ | 74 | 108 |
| Soy-SA | 80 | 96 |
| Soy-9C | 70 | 86 |
| Soy-12C | 60 | 74 |

tion is to produce droplets as small as possible. Many food products appear in the form of oil-in-water (o/w) emulsions, where the oil droplets are usually stabilized by proteins, acting as emulsifiers, along with other surfactants. Two whey proteins in combination with different phospholipids were chosen to investigate the interactions between proteins, phospholipids, and salt in such emulsion systems.[16–19]

In many textile processes, such as scouring and dyeing, it is necessary to introduce surfactants into the bath to remove oily impurities from the fibers. In these removal processes, the ability of surfactant to emulsify the oily impurities is important. The emulsifying ability of soy protein surfactants was estimated and is shown in Figure 4. It was found that the longer the hydrophobic chains possessed smaller droplet's size than other products, that is, $C_{12}$-modified soy protein has significantly greater emulsifying ability. In addition, the emulsifying ability was sensitive to the length of the hydrophobic chain presented in these soy protein surfactants. An increase in the alkyl chain length resulted in an increase in the emulsifying ability.

## Foaming properties

Foams are found in numerous applications such as foods and cosmetic products, and they are formed and stabilized in many cases by either proteins or surfactants. Gueguen et al. showed that hydrophobized peptides are very promising foaming agents and study of surface properties of new hydrophobized peptides generated from inexpensive raw materials that can make possible as a green surfactant.[20,21]

The foam height and foam stability of the soy protein surfactants are presented in Table II. It appears that the maximum foaming height was obtained for the Soy-12C and the foam height increased with the increase in alkyl chain length. The explanation to this phenomena could be, as suggested by Rosen,[22] that too short a chain probably produces insufficient cohesiveness (in the interfacial film), whereas too great a length produces too much rigidity for good film elasticity (or too low a solubility in water).

**TABLE II**
**Foaming Properties of Soy Protein Surfactants**

| | Foam height (cm) | |
|---|---|---|
| Compound | Initial | After 3 min |
| Soy-SA | 0.7 | 0.4 |
| Soy-9C | 0.8 | 0.6 |
| Soy-12C | 1.3 | 1.2 |

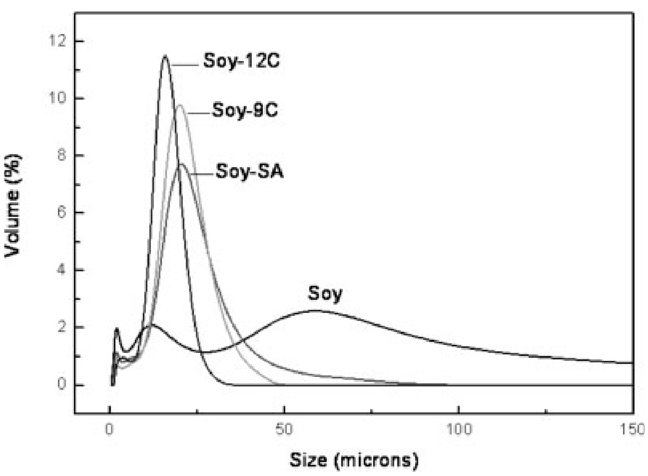


**Figure 4** Average droplet diameter of emulsions prepared at 10 mg/mL of soy protein surfactants.

## Buffer ability

In textile dyeing processes, acid or base agents are usually added to improve the softness, handle and color properties of the fabrics. However, these auxiliaries cause a pH change effect on dyes. Surfactants are used for improving the pH buffer capacity and providing a better stability under dyeing conditions.

Figure 5 shows the buffer capacity of solutions containing different soy protein surfactants. In each solution, the concentration of the surfactant is 10 g/L. This figure shows that for pure water, the pH changed quickly from 7.0 to 2.75 as the HCl amount was raised from 0 to 1.0 mL by the addition of 0.5*N* HCl. On the other hand, the results in Figure 5 indicate that the addition of the soy protein surfactants resulted in solutions that have better buffer capacities than that of pure water, especially Soy-12C.

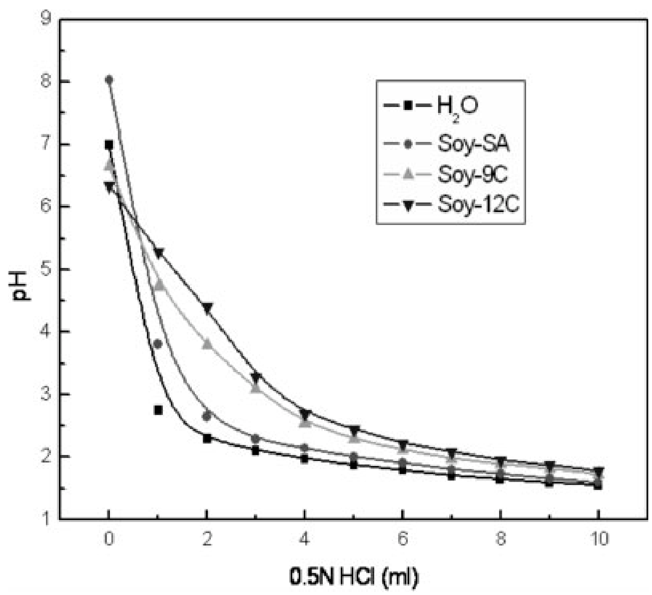


**Figure 5** Buffer ability of soy protein surfactants.

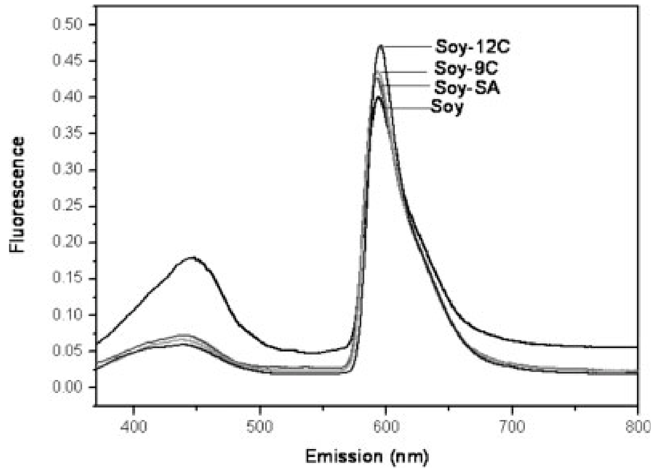


**Figure 6** Fluorescence spectra of soy protein surfactants.

## Fluorescence properties

The fluorescent probe technique is becoming increasingly popular in the study of biochemistry, molecular self-assembling systems, and macromolecule–surfactant interactions. The main spectroscopic parameters used in the characterization of micellar assemblies are excitation and emission spectral shapes, vibrational fine structure, quantum yields, and degree of polarization of emission.[23–25] These properties can be correlated with the characteristics of the microenvironments and used to predict the existence and peculiarities of the hydrophobic and aqueous domains.

The hydrophobicity of the soy protein hydrolyzate and modified soy protein surfactants was evaluated by using the emission spectra of rhodamine B.[26] Soy protein hydrolyzate was modified by hydrophobic chains of various lengths, to various degrees of attachment. As shown in Figure 6, an increase in the fluorescence intensity of the aqueous solution was observed when the numbers of hydrophobic chains that were attached to the soy protein hydrolyzate, for $C_9$, and $C_{12}$ chain lengths was increased. This phenomenon, similar to surface tension, is attributed to the enlargement of the hydrophobic portion of the soy protein hydrolyzate molecule, resulting in an increase in concentration of surfactant at the liquid surface.

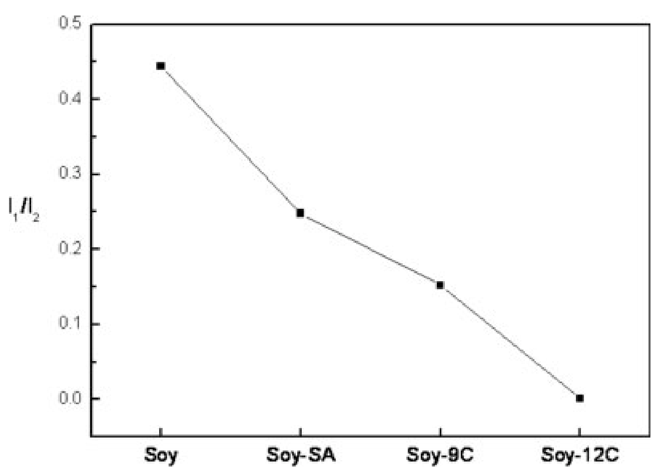


**Figure 7** The effect of chain length of the hydrophobic groups on the hydrophobicity index ($I_1/I_2$) of the soy protein surfactants.

**TABLE III**
**Whiteness of Soy Protein Surfactants**

| Compound | Whiteness (WI) 1 g/L | 2 g/L |
|---|---|---|
| Blank | 51.55 | 51.55 |
| Soy-SA | 57.16 | 50.15 |
| Soy-9C | 59.26 | 55.37 |
| Soy-12C | 63.55 | 58.24 |

Figure 7 showed that the unmodified soy protein hydrolyzate caused a slight decrease in the $I_1/I_2$ ratio from 0.445 of the aqueous solution to 0.002 of the soy protein surfactant solution, where $I_1$, $I_2$ are the fluorescence intensity of the soy protein surfactant and probe, respectively.[27] The ratio between peak 1 ($I_1$) and peak 2 ($I_2$) in the emission spectra of rhodamine B can be used as an index to hydrophobicity. In a hydrophilic medium, this ratio is high, and in a hydrophobic medium this ratio is low. The attachment of hydrophobic groups to the soy molecule increases its hydrophobicity, it appears that increasing the chain length causes a significant reduction in the $I_1/I_2$ ratio for soy protein surfactant.

## Application in cotton bleaching

Usually, there are two steps necessary to obtain textile quality fibers. Bacterial, chemical, or enzymatic retting is the first step, and bleaching is an additional step. Chemical retting is a rapid process, but the use of chemicals may create environmental or waste-disposal problems. Bleaching or similar treatments improve the appearance of bast fibers. Most of these treatments include peroxide bleaching, alkali and enzyme treatments, and biobleaching.[28]

The ability of soy protein surfactant to improve the whiteness of cotton fabrics in a hydrogen peroxide bleaching system are shown in Table III. It is that the presence of soy protein surfactants increase the effects of oxidation on bleaching baths in the process and prepare the more purified fabrics for the next processes of dyeing or printing.

## CONCLUSIONS

A novel series of soy protein surfactants with surface active properties were prepared by the reaction of soy protein hydrolyzate and alkyl succinic anhydride. These novel compounds were found to exhibit good surface activities, including surface tension, wetting power, foaming, and emulsifying ability. The surface activity of the investigated compounds in aqueous solutions increases with the increase of the alkyl chain length in the hydrophobic fragment of surfactants. Good surface properties are displayed by surfactant with dodecyl groups in the molecule. It is also indicated that the soy protein surfactants impart the whiteness effect for cotton fabrics are referred to as bleaching surfactants.

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