Caseinate Based Biodegradable Films with Improved Water Resistance

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ABSTRACT: Proteins are considered as an interesting alternative to traditional synthetic polymers in packaging applications. Sodium caseinate based films can be used in such area, but some of their properties need to be improved. The objective of this work was to improve water resistance of caseinate based films plasticized with triethanolamine (TEA). First, plasticized films were crosslinked by formaldehyde (HCHO) or by electron beam irradiation. The crosslinking efficiency was correlated to the decrease of protein solubility in water determined from a 280 nm absorbance method. The comparison between the two crosslinking methods showed that formaldehyde crosslinking was significantly more efficient than irradiation. The HCHO crosslinking technique was selected for the following of the study. Nevertheless, even for highly crosslinked samples, the plasticizer exuded out of the film. A second part of this work was focused on the effect of surface modification on plasticizer exudation in TEA plasticized caseinate films. Considering that silicone grease coating onto the film surface was able to control TEA exudation, surface modifying additives (SMA) based on NaCAS and organo-silicones were used to modify films surface properties. Surface wettability and energy were determined from contact angle measurements. TEA exudation ratios in water were also monitored for films containing SMA. SMA were less efficient in controlling TEA exudation rates but could significantly reduce surface energy to 42 mJ m^2. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 117: 1828–1836, 2010

Key words: caseinate film; triethanolamine; platicizer; exudation; silicone; organosilicone; surface energy

INTRODUCTION

Among the natural polymers that could be used in packaging applications, proteins are available for easy replacement of conventional synthetic polymers. Indeed proteins can be considered as thermoplastic hetero-polymers based on 20 amino-acid monomers that have different side group attached to the central carbon. These side groups can be chemically or enzymatically modified to improve film properties. Protein films also generally exhibit good mechanical properties and also good barrier properties to gases such as O_2 or CO_2. The production and properties of films and coatings based on proteins have been comprehensively reviewed by Gontard and Guilbert[1] or Krochta et al.[2] Many proteins have film forming abilities and have been used for the manufacture of packaging materials with interesting properties: wheat gluten, corn zein, soybeans, collagen, ovalbumin, whey protein, casein, egg proteins, myofibrillar proteins, etc. Among these proteins, sodium caseinate (NaCAS) was selected for the manufacture of films and coatings. NaCAS is a commercially available protein obtained by acid precipitation of casein, the main protein in cow's milk. This protein is obtained with good purity (up to 95%) and present thermoplastic and film forming properties due to its random coil nature and its ability to form weak intermolecular interactions i.e. hydrogen, electrostatic and hydrophobic bonds.[3,4] Considering their transparency, biodegradability and good technical properties (high barrier for gases like O_2),[5] caseinate based films can find applications, not only in packaging,[6,7] but also in edible or protective films and coatings[8,9] or in mulching films.[10] Such films are easily obtained from casting aqueous solutions of sodium caseinate. In a previous work[11] caseinate based films were prepared with improved properties as close as possible to available packaging films based on synthetic polymers like polyethylene[8] or plasticized PVC.[12] Caseinate films were plasticized with triethanolamine (TEA) to confer interesting mechanical properties to the material like good elongation at break (about 200%) and a rather low tensile strength (less than 8 MPa). The incorporation of a minimal content of plasticizers is needed to avoid brittleness and to increase extensibility and flexibility.[13,14] By decreasing intermolecular forces

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between polymer coils, the plasticizer causes an increase in material flexibility and conversely a decrease in the barrier properties due to the augmentation of the free volume. Plasticizer efficiency is mostly governed by its molecular weight and polarity and its ability to reduce intermolecular hydrogen bonding while increasing intermolecular spacing.

Further, to increase mechanical strength and to improve water resistance, caseinate films can be crosslinked: the occurrence of covalent bridges between protein chains allows water-insoluble three-dimensional network to be achieved. The most commonly used techniques for crosslinking protein materials include chemical crosslinking with reactants like formaldehyde[15] or glutaraldehyde,[16,17] enzymatic crosslinking[18,19] with transglutaminases or peroxydases and physical treatments such as irradiation. Radiation of proteins (electron beam, γ irradiation) is a way to induce their crosslinking and to improve their performances[20] like mechanical strength. The radicals formed during irradiation promote a binding between adjacent molecules forming a three-dimensional network. Because of the limited availability of transglutaminases and peroxydases, their high cost and their limited efficiency at macromolecular level, enzymatic crosslinking is not extensively used in the manufacture in films and coatings.

The objective of the present study was to investigate the crosslinking effects of formaldehyde and electron beam irradiation on the water solubility of caseinate based films. Crosslinking caséinate based films with formaldehyde was performed as described in a previous work.[11] The comparison between the two techniques showed that using HCHO was a most efficient way to promote crosslinking and to reduce protein solubility.

In a second part of this article, effect of surface modification on plasticizer exudation in plasticized NaCAS films was studied. Indeed, in spite of crosslinked protein material being water insoluble, plasticizer exuded out of the film, even for highly crosslinked samples. The exact fraction TEA plasticizer that exuded out of the films was monitored by the voltammetric method. According to the massive exudation of TEA observed, solutions were proposed to reduce TEA exudation. To increase surface hydrophobicity, silicone wax was first coated onto the film. Then, regarding the results, to see the effect of permanent surface modification on TEA exudation, silicone based surface modifying additives (SMA) were prepared and added in NaCAS plasticized films formulations and their effect on plasticizer exudation was monitored. Indeed, polysiloxane and particularly polydimethylsiloxane (PDMS) are interesting polymers since they present unique properties including low glass transition temperature (T_g about $-120°C$), high chain flexibility, water repellency (hydrophobicity), good blood compatibility and especially low surface energy. Previous studies[21] have shown that due to their very low surface energies and low solubility parameters, when copolymerized or blend with other organic polymers, PDMS tend to migrate to the polymer surface. To obtain permanent surface modification it is possible to use siloxane copolymers with organic moieties. In such copolymers, also called SMA the organic component of the siloxane copolymer provides miscibility with the base polymer (bulk) while siloxane segments go to the polymer surface. Only a small quantity of SMA will significantly modify the surface energy leading to blends with completely silicone-like surface properties and keeping bulk properties of the initial material almost intact. In silicone based SMA, the organic part of the copolymer affords miscibility with the base polymer while siloxane segments migrate to the material surface. In the present work two SMA were synthesized from sodium caseinate and two different telechelic polysiloxanes: (i) a techelic organoamine terminated PDMS oligomer and (ii) a telechelic organoepoxy terminated PDMS oligomer. In a second step the SMA was incorporated in plasticized NaCAS based films to obtain permanent surface modification. Surface properties of the SMA but also of the NaCAS based films blended with SMA were investigated by water contact angle measurements. Role of the type and composition of silicone additive on the surface properties of NaCAS based films were determined.

EXPERIMENTAL SECTION

Materials and reagents

Sodium caseinate (NaCAS) was purchased from Eurial (France). Its composition according to manufacturer was: proteins 90.2%, water 5.7%, minerals 3.5%, fat <1%. TEA is of analytical grade (purity 99+%, Acros Organics). Aminoalkyl functional polydiméthylsiloxane (Tegomer A-Si 2322) and epoxy-functional polydimethyl siloxane (Tegomer E-Si 2130) were gently supplied by Degussa (Goldschmidt). According to the manufacturer Tegomer A—Si 2322 is composed of 30 monomer units ($Me_2Si—O—$) and Tegomer E-Si 2130 is composed of 10 monomer units ($Me_2Si—O—$). All other reagents including magnesium nitrate (99+%), sodium azide (99%), sodium bicarbonate (99%) and formaldehyde (HCHO, 37 wt % solution in water stabilized with 10–15% methanol) were obtained from Acros Organics and used as received.

Préparation of the initial caseinate solution

An aqueous mixture (A) of sodium caseinate (50 mL, 5% w/v) and TEA (TEA/protein ratio

w/w = 50%) was magnetically stirred at 800 rpm for ~12 h at room temperature to obtain a homogeneous solution. The protein was crosslinked with formaldehyde using different HCHO/NaCAS molar ratios. Molar ratios were calculated in consideration of the 12.4 mol of potentially reactive amino acid residue, i.e., lysine (Lys), contained in 1 mol of NaCAS.[22] The ε-amino group of lysine was considered the primary reactive site between proteins and aldehydes.[23] The pH was controlled by TEA to 8.6 without the use of any buffer. The protein/formaldehyde dispersion was left to react with gentle stirring at room temperature for 6 h. Films were identified as T50 when plasticized with 50% w/w TEA followed by the HCHO/NaCAS ratio into brackets. Ex: T50(1.37)

Irradiation

The films were irradiated at room temperature by electron beam using "Bolloré division films plastiques" equipment. Irradiation doses varied from 20 to 60 kGy.

Water solubility

The solubility of sodium caseinate was determined by assaying for protein using the 280 nm absorbance method. NaCAS films (20 × 20 mm; ~100 mg) were immersed in distilled water (75 mL) at 20°C and magnetically stirred at 250 rpm. From absorbance at 280 nm of the supernatant the remaining soluble protein was determined. The solubility is expressed in weight percentage of initial amount of dry casein in the film.

Absorbance at 280 nm of NaCAS solutions in distilled water was first measured. It was also checked that TEA absorbance was negligible at 280 nm. The calibration straight line ($y = 1.457x + 0.016$) of the absorbance of NaCAS solutions versus NaCAS concentration is obtained with a correlation coefficient $r = 0.9981$. The calculated molar extinction coefficient was $\varepsilon = 32{,}920$ L mol^{-1} cm^{-1}. NaCAS content in film can be determined with an accuracy better than 5%. The solubility of TEA plasticized films crosslinked with formaldehyde was determined after 2, 5, 10, 20, 40, 60 min, and 24 h from the 280 nm absorbance of the supernatant.

SMA preparation

SMA-E

An aqueous mixture of sodium caseinate (20 mL, 5% w/v) was magnetically stirred at 800 rpm for ~12 hours at room temperature to obtain a homogeneous solution. The tegomer E-Si2130 solution was mixed with the NaCAS solution at a 2 : 1 ratio of epoxyde group in tegomer E-Si2130 to NH_2 of NaCAS. The protein/tegomer dispersion was left to react with gentle stirring at room temperature for 6 h.

SMA-A

An aqueous mixture of sodium caseinate (20 mL, 5% w/v) was magnetically stirred at 800 rpm for ~12 hours at room temperature to obtain a homogeneous solution. The pH of the solution was adjusted to 9.5 with a 1 *M* water solution of sodium hydroxide. Formaldehyde was added to the NaCAS solution with a 10 : 1 ratio of HCHO to NH_2 of NaCAS. In the same time, tegomer A-Si2322 was joined to the solution with a 1 : 1 ration of the tegomer to the NH_2 of NaCAS. The protein/formaldehyde/tegomer dispersion was left to react with gentle stirring at 60°C for 6 h. For both SMA, solutions were washed three times with *n*-hexane to remove the excess of unreacted tegomer.

Preparation of NaCAS and NaCAS/SMA films

Caseinate films were then obtained by the casting method: the film forming solution was spread onto a polystyrene plate and after the excess water was evaporated the film was peeled off. Samples were kept in a closed tank and maintained at 53% relative humidity with a saturated solution of $Mg(NO_3)_2$. For NaCAS films, the initial solution (A) is directly spread onto the plate. For NaCAS/SMA films, the water solution of each SMA was mixed with the initial NaCAS solution (A) before spreading onto the polystyrene plate. Films obtained from tegomer E-Si2130 and tegomer A-Si2322 were identified as NaCAS-E and NaCAS-A respectively. In Figure 5, the reference NaCAS film was obtained from the initial solution (A) containing formaldehyde (5 : 1 ratio of HCHO to NH_2 of NaCAS) and blended with an aqueous solution of sodium caseinate (20 mL, 5% w/v) to respect the same protocol applied to the NaCAS-A and NaCAS-E films.

TEA exudation rate

TEA is known to have the ability to chelate certain metallic ions in highly alkaline medium (pH > 12), such as the ferric ion. In this work TEA concentration was obtained using differential pulse voltammetry based on the measurement of the concentration of TEA-Fe^{3+} complex.[24,25] The NaCAS films (20 × 20 mm; ~100 mg) were immersed in distilled water (75 mL) at 20°C and magnetically stirred at 250 rpm for 2, 5, 10, 20, 40 min, and 24 h respectively. A 100 μL of the supernatant was then transferred to the voltammetric cell and diluted with 10 mL 1*M* NaOH solution as supporting electrolyte. A 15 μL to 290 μL of 0.5 g L^{-1} solution $NH_4Fe_{III}(SO_4)_2$, 12 H_2O was

TABLE I
Samples Preparation for Voltammetric Analysis

Immersion time in distilled water	1*M* NaOH solution	0.5 g L^{-1} $NH_4Fe_{III}(SO_4)_2$, 12 H_2O solution
2 min	10 mL	40 μL
5 min	10 mL	40 μL
10 min	10 mL	40 μL
20 min	10 mL	50 μL
40 min	10 mL	50 μL
24 h	10 mL	70 μL

added into the electrochemical cell (see Table I). The solutions were de-aerated with nitrogen gas for 10 min and an inert atmosphere (N_2) was maintained over the solutions during measurements. Electrochemical experiments were performed using three electrodes. The voltammograms of these solutions were obtained with a micro autolab polarographic analyzer Metrohm piloted by the GPES software. The working electrode was a hanging mercury-drop electrode (HMDE), the reference electrode was Ag/AgCl/Cl^- and the counter electrode was a Pt electrode. The setting parameters were as follows: pulse height 50 mV, step potential 0.0012 V, potential −1.2 to −0.7 V versus Ag/AgCl/Cl^-. It was assumed that the TEA was fully converted to the TEA-Fe^{3+} complex. The amount of TEA was determined from the TEA-Fe^{3+} complex peak intensity. The calibration straight line (y = 4E-8x + 2E-9) of the TEA-Fe^{3+} peak intensity (A) versus TEA concentration (mg L^{-1}) was obtained with a correlation coefficient r = 0.9897.

Wettability measurement

A Digidrop GBX model DS goniometer equipped with a digital camera was used for the contact angle measurements. The computer software windrop++ was used to calculate contact angles and surface energies. The sessile drop method was used to characterize the wettability of the caseinate based films. The contact angle θ was determined by placing at least five drops (5 μL) of the test liquid on the film. The energetic parameters of the surface free energy (γ_s) and its dispersive (γ_L^d) and non dispersive terms (γ_L^{nd}) of the film surface were calculated from the respective contact angles of water and diiodomethane. The energetic parameters of water and diiodomethane are detailed in Table II.

TABLE II
Energetic Parameters of the Two Testing Liquids

Testing liquid	γ_L (mJ m^{-2})	γ_L^d (mJ m^{-2})	γ_L^{nd} (mJ m^{-2})
Water	72.8	21.8	51
Diiodomethane	50.8	50.8	0

Imaging by scanning electron microscopy SEM-FEG

Imaging of both chemical fouling and microorganisms was obtained with an electron microscope with field emission (JEOL JSM 6301 F –9 kV). The samples were dried in a dessicator under vacuum.

RESULTS AND DISCUSSION

Protein solubility: Effect of crosslinking with formaldehyde

TEA plasticized NaCAS films with various ratios of HCHO crosslinker were immersed into distilled water. The water solubility of sodium caseinate (percentage of initial dry weight of NaCAS in films) versus immersion time is shown in Figure 1. For the unreacted NaCAS film, the NaCAS specific solubility increased rapidly and after 10 min the film was completely dissolved. However, for the crosslinked films, the solubility remained less than 6% after 40 min of immersion in water, whatever the HCHO/NaCAS ratio, indicating the water resistance of crosslinked samples. Protein specific solubility ranged from 11.4% for the lowest HCHO/ε-NH_2 molar ratio (0.67) to 1.8% for the highest ratio (6.75). Figure 1 clearly points out that the higher the HCHO/ε-NH_2 ratio the lower is the solubility.

Protein solubility: Effect of electron beam irradiation

Radiation of proteins also induce their crosslinking leading to the formation of a three-dimensional network. As observed on Figure 2, protein solubility decrease with the increment of radiation dose except after 24 h immersion in water. Radiations are particularly efficient on protein solubility for short

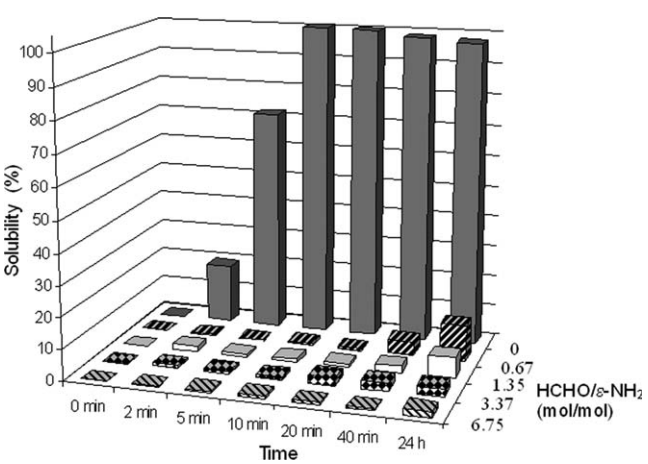


Figure 1 Protein solubility of 50% TEA plasticized films in water from 280 nm absorbance method versus time for HCHO/ε-NH_2 molar ratios reported on the right hand side.

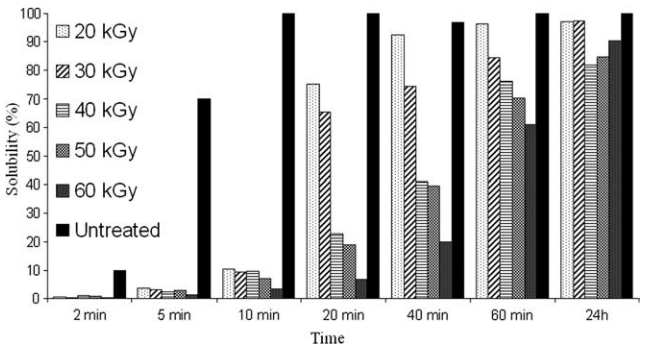


Figure 2 Protein solubility of 50% TEA plasticized films versus irradiation dose and immersion time in water.

immersion times. After 20 min immersion in water, less than 7% of the initial amount of protein is solubilised for films irradiated at 60 kGy compared to about 100% for the reference sample. Nevertheless, for all samples and even for the most irradiated ones the solubility increase with the immersion time in water. After 24 h immersion, the solubility increased over 80% for all samples. The protein degradation caused by high irradiation doses should explain the slight difference observed after 24 h immersion in water and why films irradiated at 40 kGy were the less soluble. The degradation was concomitant to the crosslinking and caused protein chain fragmentation. Then small fragments obtained could be more easily extracted by water.

The comparison between Figures 1 and 2 showed that protein crosslinking with formaldehyde was significantly more efficient than with electron beam irradiation. The HCHO crosslinking technique was selected for the following of the study.

TEA exudation

TEA (50%) plasticized NaCAS films with various ratios of crosslinker were immersed into distilled water to determine the quantity of TEA lost by the films. The exudation ratio of TEA (dry weight percentage of TEA initially present in films) versus immersion time is shown in Figure 3. As suspected and described elsewhere,[11] the water soluble plasticizer exuded out of the film: after 10 min immersion in distilled water about 80% of the TEA migrated out of the film, whatever the formaldehyde content. For all the NaCAS films, the whole TEA exuded out of the film after 24 h in water. Nevertheless, for short time of contact with water (2 min), the plasticizer migration decreased with increased crosslinker content, indicating a steric hindrance to TEA exudation. Because TEA is a water-soluble plasticizer it migrates easily out of the film. Indeed, for some authors, global solubility is expressed without taking into account the plasticizer content in the dry matter of the film,[26] considering that the water soluble plasticizer is automatically exuded from the film during the water solubility test.

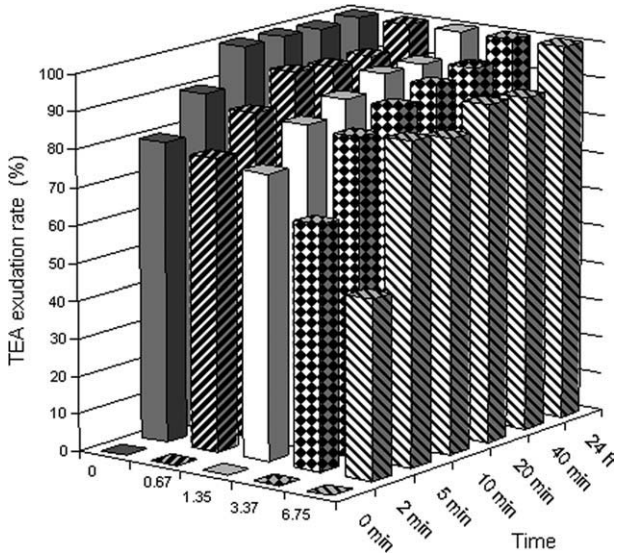


Figure 3 TEA exudation rate for 50% TEA plasticized films from voltammetric analysis versus water immersion time and HCHO/ε-NH_2 molar ratios reported on the front side.

Silicone grease coating: Effect on TEA exudation ratio

Silicone grease was first coated onto the plasticized NaCAS films to make a hydrophobic layer that should prevent TEA exudation. Figure 4 shows TEA migration ratio of silicone grease coated films compared to uncoated ones. Two kinds of 50% TEA plasticized films were tested, one crosslinked with HCHO/—NH_2 ratio of 1.35 the other with HCHO/—NH_2 ratio of 3.37. Silicone grease coating onto plasticized NaCAS films was able to control

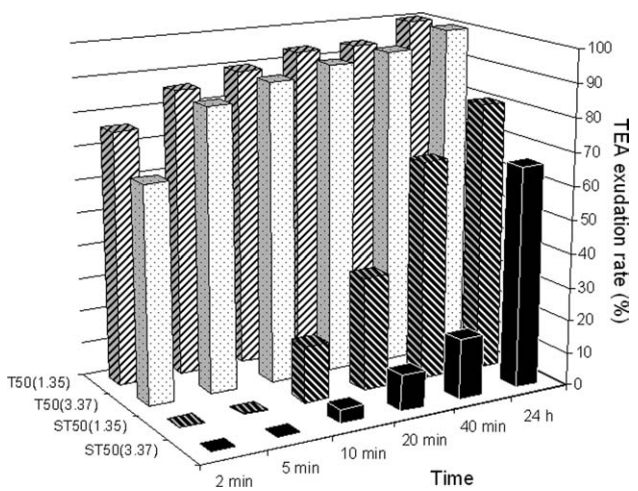


Figure 4 TEA exudation rate for crosslinked T50 films as a function of water immersion time. HCHO/—NH_2 ratio is noted into brackets. The first letter S means the film was coated with silicone grease.

plasticizer exudation. As expected, silicone grease coated samples identified as ST(50) showed lower TEA exudation ratios than reference films: for short times of water immersion, less than 1% of TEA migrated out of the films. Nevertheless, after 10 min immersion, small quantities of TEA were extracted by the water, and the plasticizer exudation ratios gradually increased until maximum values close to the ratios of uncoated samples after 24 h immersion. For T50 films crosslinked with HCHO/—NH_2 ratio of 1.35, after 20 min of water immersion only 34% of TEA was extracted for the silicone coated film compared to 96% for the uncoated one. After the same time of immersion, T50 films crosslinked with HCHO/—NH_2 ratio of 3.37 and coated with silicone grease presented an exudation ratio of only 11%, which is clearly lower than the 73% observed for uncoated films. Until 40 min, TEA migrations were significantly lower in coated samples compared to the uncoated ones.

Considering the polar nature of TEA, the hydrophobic layer composed of the silicone grease constitutes an obstacle to the passive exudation of TEA for short times of immersion. Moreover, the silicone layer should also hinder water penetration into the film making TEA extraction by the surrounding water more difficult.

Nevertheless, after 24 h immersion, TEA migration values obtained with silicone coated films increase and almost reach the observed values for uncoated samples. This could be correlated to the nonpermanence of the coating: the silicone layer is not covalently bounded to the protein film and can also be extracted by water under stirring. Then the resulting uncontinuous layer on the film surface is not able to prevent anymore TEA leakage.

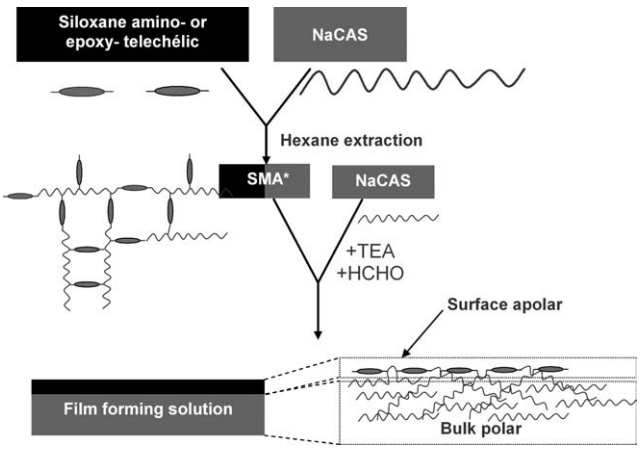
Taking into consideration these primary results and to obtain permanent surface modification, silicone based oligomers were covalently linked to NaCAS proteins according to the procedure descried in the experimental part.

Films with SMA: Surface characterization

The SMA technology was selected to overcome TEA migration problem. Such SMA are generally used in small quantities: only a low percentage (less than 2% in most cases) of the additive to the bulk polymer can significantly improve surface properties. The surface is "permanently" modified because the polymer segments of interest for surface modification are covalently linked to the bulk polymer. The two SMA tested here, SMA-A and SMA-E were based on polysiloxanes, amino- and epoxy-telechelic respectively that reacted with NaCAS proteins. For each film, the SMA was blended to the NaCAS solution containing the plasticizer and the solution was finally poured onto the plastic plate. The objective was to create hydrophobic domains on the film surface through SMA migration during film fabrication. Change in the surface-functional groups results in a change of the surface wettability, which can be monitored by contact angle measurements. Indeed, the molecular approach of the surface free energy leads to the consideration of the effect of the dispersive and the nondispersive forces, corresponding to long-range Lifschitz-Van der Waals interactions and to short-range polar interactions, respectively. Although the nondispersive component could be expressed by a donor and an acceptor part, our analysis was performed considering the γ_s^d and γ_s^{nd} terms of the γ_s and using the following correlation of the contact angle with the surface energy parameters: $\gamma_L(1+\cos\theta) = 2\sqrt{\gamma_L^d\gamma_S^d} + 2\sqrt{\gamma_L^{nd}\gamma_S^{nd}}$. Considering the contact angles θ_1 and θ_2 obtained with the two reference liquids respectively L_1 and L_2 with known γ_L^d and γ_L^{nd} it is possible to calculate γ_s, γ_s^d, and γ_s^{nd} by solving a system of two equations. Table III gives the surface energy parameters calculated for Plasticized NaCAS reference film compared to those of films containing SMA-A and SMA-E identified as NaCAS-A and NaCAS-E respectively. The surface of the reference film is relatively polar according to the measured water contact angle $\theta_w = 51°$. For both films containing SMA, NaCAS-A, and NaCAS-E, the surface is more hydrophobic, showing water contact angles of 92° and 82°, respectively. This is correlated to the decrease of the surface energy γ_s which can mainly be attributed to the lowering of the non dispersive component. This is due to the silicone part of the SMA that migrates to the film surface during manufacture: hydrophobic domains are then created on the surface. The incompatibility of the siloxane segments with the bulk polymer is correlated to the fact that there are no specific interactions between the silicone segments and the protein chains that would make the variation of enthalpy of mixing and then the mixing free energy favorable to the mixing of protein and silicon parts. Nevertheless, the protein segments of the SMA would ensure a relative compatibility with the protein in the films. According to these considerations, the behavior of the SMA blended with NaCAS is summarized in Scheme 1.

TABLE III
Wetting Characteristics of NaCAS Based Films. θ_w Is the Contact Angle of the Water Drops with Films

		Wetting characteristics		
Film	θ_w	γ_S (mJ m^{-2})	γ_S^d (mJ m^{-2})	γ_S^{nd} (mJ m^{-2})
T50(3.37)	51°	54	33.7	20.3
NaCAS-A	92°	42.1	41.6	0.5
NaCAS-E	82°	45.1	42.8	2.3



Scheme 1 Preparation method for SMA modified films.

Films with SMA: Effect on TEA exudation ratio

TEA exudation ratio is monitored for NaCAS-A and NaCAS-E versus water immersion time and compared to reference film without SMA (Fig. 5). After 24 h, almost 100% of the initial amount of plasticizer is extracted by the water for all the samples. A slight difference can eventually be noticed for 2 min of water immersion, where NaCAS-A samples present lower TEA exudation ratios (22%). Compared to the results obtained with silicone grease coated samples, SMA are less efficient in controlling TEA exudation ratio and the SMA effect is only observed for short immersion times (2 min). The nonpermanence of the surface effect should be correlated to several factors. First, the SMA would not be compatible enough with the protein bulk even if it contains protein segments. Therefore it could be extracted by the surrounding water under stirring. Secondly, when exposed to a very polar media, the apolar silicone segments could rearrange themselves to present polar protein segments on the surface, contrary to the scheme 1 representing an homogeneous hydrophobic layer on the surface of the film. Lastly, the hydrophobic layer created on the film surface with silicone parts of the SMA is not continuous and water could easily penetrate into the film to extract TEA or TEA can also exude passively through preferential ways. Contact angles have been measured on the surface of SMA-modified films after immersion in water or in *n*-hexane for several hours. Results are not represented here, but in all cases the surface was still hydrophobic (and sometime more), indicating that the silicone layer was not extracted by surrounding solutions.

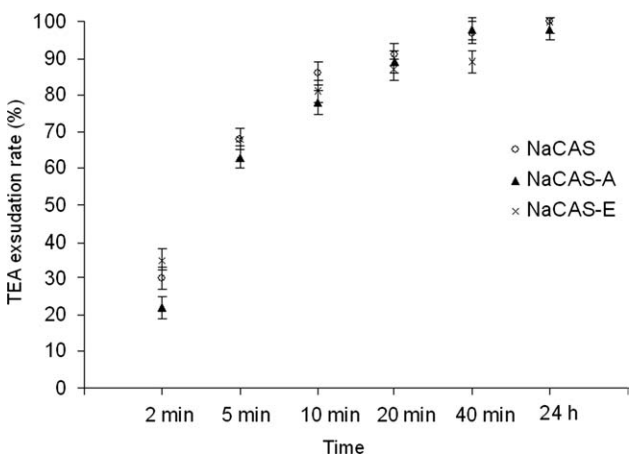


Figure 5 TEA exudation rate for NaCAS based films from voltammetric analysis versus water immersion time.

Films with SMA: Further surface characterization and SEM micrographs

To find an explanation to the nonpermanence of surface modification with the two silicone based SMA, the wetting characteristics were performed for longer time of contact between reference liquids and material surface, respectively after 5 and 10 min. Values are reported in Table IV and compared to the initial values. As suspected, for both samples, the water contact angle decreases when contact time increase. For the NaCAS-A sample, the θ_w decreased from 92° to 63° after 10 min contact between water and the material surface, indicating that the surface is becoming more polar. The same phenomenon is observed for the SMA-E sample were the contact angle with water drops decreased from 82° to 47° after 10 min. Considering the surface energy, this can be explained by an increase in the non dispersive term of the surface free energy, as mentioned in Table IV, witch confirm that the surface is becoming more polar. Then, it can be suggested that the surface modification is not permanent and that apolar silicone segments would rearrange themselves to present polar protein segments on the surface of the films when exposed to polar media.

Scanning electron micrographs of the samples surfaces are shown in Figure 6, which further attests that the surface is more heterogeneous when the film contains SMA. The reference sample exhibited a relative homogeneous surface with minimal or no holes on it [Fig. 6, T50(3.37) ×2000 and ×5000]. On the other hand, holes or phase separation can be observed on the surface of samples containing SMA, particularly for SMA-A samples where some drops can be seen attesting that the material is not so homogeneous (Fig. 6 SMA-A ×5000). These SEM observations suggest that the hydrophobic layer created on the film surface with silicone parts of the SMA is not continuous: water can easily penetrate into the film to extract TEA and/or TEA can also exude passively through preferential ways.

TABLE IV
Wetting Characteristics of NaCAS Based Films after 5 and 10 Min Contact Between Reference Liquids and Material Surface. θ_w Is the Contact Angle of the Water Drops with Films

		Wetting characteristics			
Film	Time of contact	θ_w	γ_S (mJ m^{-2})	γ_S^d (mJ m^{-2})	γ_S^{nd} (mJ m^{-2})
NaCAS-A	0 min	92°	42.1	41.6	0.5
	5 min	68°	38.3	28.3	9.9
	10 min	63°	41.4	28.3	13.1
NaCAS-E	0 min	82°	45.1	42.8	2.3
	5 min	48°	52.4	34.9	17.7
	10 min	47°	54	35.3	18.7

CONCLUSION

The main target of the present work was to obtain NaCAS films with improved water resistance and permanent plasticization. Enhanced tensile properties were obtained by selecting a convenient plasticizer like TEA. Chemical crosslinking of NaCAS

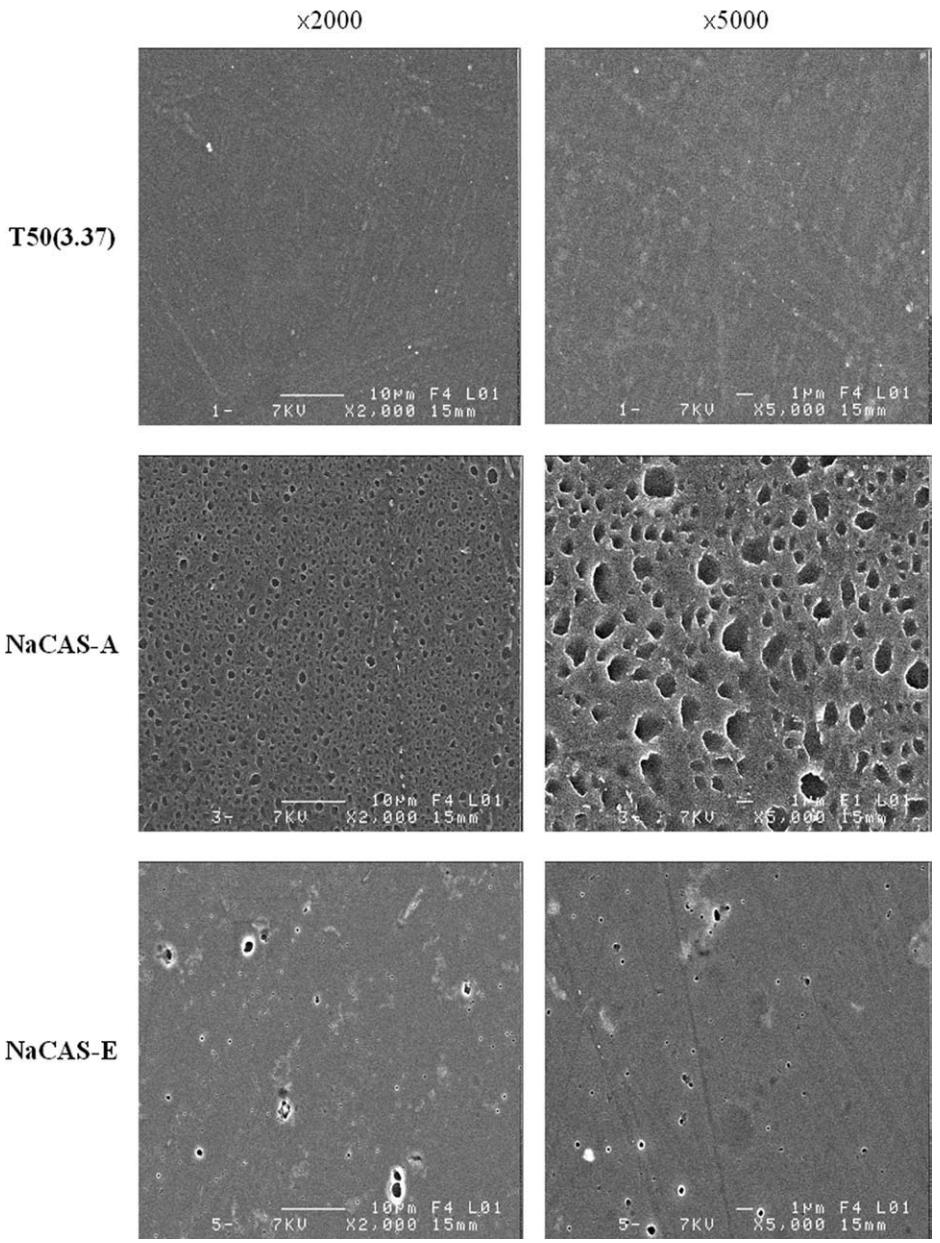


Figure 6 SEM micrographs of the surface of T50(3.37), NaCAS-A and NaCAS-E samples at 2000× and 5000×.

with formaldehyde is an efficient way to overcome water sensitivity of the protein film. Nevertheless, electron beam was also shown to be efficient for controlling protein solubility especially for short time of water immersion. This technique should thus be used in particular cases to crosslink proteins in absence of any bifunctional chemical compound that could represent a problem in some particular applications (food contact or skin contact).

During this study, it was also shown that crosslinking was not efficient for controlling the plasticizer exudation out of the film when immersed in water. Then to reduce the plasticizer extraction by water, plasticized caseinate films were coated with silicone grease. This technique was efficient to partially control the TEA exudation out of plasticized films particularly for short time of immersion in water, typically less than 10 min. To obtain permanent surface modification, silicone based oligomers were covalently linked to NaCAS proteins to prepare SMA. Two SMA were tested, SMA-A and SMA-E based on polysiloxanes, amino- and epoxytelechelic respectively. Surface of NaCAS based films blended with SMA was investigated by water contact angle measurements. It was shown that the use of SMA greatly reduces the films surface energy but has no significant effect on the plasticizer exudation. This can be correlated to surface heterogeneity and to the nonpermanence of surface hydrophobization through polar/apolar segments rearrangements.

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