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ORIGINAL CONTRIBUTION

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Properties of complexes and particles of gelatin with ionic surfactants

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Abstract The properties of soluble gelatin-ionic surfactant complexes and insoluble particles were evaluated. It was found that colloidal particles of gelatin A – cationic surfactant (dodecyltrimethylammonium bromide, DTAB, and cetyltrimethylammonium bromide, CTAB) were formed. Binding isotherms showed that these particles are obtained above the CMC of each surfactant, while cooperative binding takes place.

Surface tension measurements conducted for both gelatin/DTAB and gelatin/anionic surfactant, SDS (sodium dodecyl sulfate) showed a break in the curve describing surface tension vs number of bound surfactant molecules, (v) at concentrations below the CMC of each surfactant alone. This break, which is attributed to CMC 1, is observed at the same number of bound surfactant mol ecules $v \sim 2$ for both gelatin/surfactant couples.

Contact angle measurements showed that the maximal hydrophobicity of the gelatin-surfactant particles is obtained at the same concentration range in which the precipitation occurs. It was also found that the hydrophobicity of gelatin-SDS particles, is higher than that of the gelatin-cationic surfactants, due to a different composition of the resulting particles.

The zeta potential of the particles indicated charge neutralization and even charge reversal for gelatin-CTAB at high surfactant concentration.

Key words Gelatin – surfactant – sodium dodecyl sulfate – dodecyltrimethyl-ammonium bromide – cetyltrimethylammonium bromide – binding – surface tension – hydrophobicity – zeta potential

Introduction

Gelatin is widely used in various applications, amongst them food emulsions, photographic emulsions, microcapsules and cosmetic formulations. Gelatin has unique properties, which are particularly suitable for use in photographic emulsions. In these emulsions, the continuous medium should be transparent, and with such rheological properties that will allow uniform coating. In addition, the gelatin functions as a protective colloid, which affects the formation and crystal growth in the system [1–3]. Many of the systems (including photographic emulsions) contain, in addition to gelatin, various types of surfactants. As shown in several publications and patents, the physical and photographic characteristics of silver halide emulsions strongly depend on the surfactants which are present during the addition of gelatin. In particular, the added

surfactants lead to formation of a uniform coating layer [4]. In such systems, anionic and nonionic surfactants, with linear or branched alkyl chains, are used [5]. For example, Bertramini and Baldassarri [6] suggested the formation of photographic emulsions with nonionic surfactants, such as sorbitan esters, with HLB over 10, and anionic surfactants with HLB over 20. The possible interactions of surfactants with the gelatin may affect the functional properties of both gelatin and surfactant, and may be used, at specific conditions, to achieve unique dispersion systems, such as microcapsules [7–10]. In particular, the influence of surfactants on the secondary structure is of great importance in photographic science [11]. The interactions between surfactants such as sodium dodecyl sulphate (SDS), affect both the rheological properties and adsorption behavior of the gelatin.

In general, it was found that gelatin can form soluble complexes, or may precipitate as a result of interaction with ionic surfactants such as SDS or cetyltrimetylammonium bromide (CTAB) [1, 12–14]. Whitesides and Miller [15] found that the complex formation led to aggregation of the surfactant molecules, even at concentrations below the CMC. They concluded that the interaction between SDS and photographic grade gelatin, involves the formation of micelles attached to the polymer in a necklace and bead model, as suggested for SDS interaction with synthetic polymers. The surface tension vs surfactant concentration presented a typical behavior, in which the first break in the curve is obtained ("CMC (1)") due to attached aggregates to the gelatin. Miller et al. [16] reported that gelatin interacts with SDS through electrostatic attraction of cationic residues with the anionic surfactant headgroup, and through hydrophobic attraction of non-polar and aliphatic residues with the exposed hydrocarbon tails of the surfactants. Knox and Wright [17] studied the regions of the complex precipitation and solubilization, and concluded that at pH below the isoelectric point the complex precipitation occurs when only half of the cationic sites of the protein are occupied by the adsorbed surfactant molecules. Solubilization of the precipitated complex is achieved by adsorption of additional SDS with the hydrophillic groups oriented toward the water. Chen and Dickinson [18] studied the flocculation of gelatin-stabilized emulsions and gelatin precipitation in presence of an anionic surfactant, and concluded that maximal charge neutralization is obtained at the maximal degree of flocculation. The electrophoretic mobility of gelatin-anionic surfactant was dependent on surfactant concentration, causing charge reversal of the positively charged gelatin into a negatively charged complex [19].

Interaction of gelatin with cationic surfactants was reported by several investigators [12, 20–22]. Fruhner and Kretzschmar [21] found that cationic surfactants show much weaker interactions with gelatin than anionic surfactants of comparable alkyl chain length, and that the number of bound molecules is strongly dependent on the pH. Furthermore, different binding was reported for cationic surfactants, having different hydrophobic chain length, emphasizing the role of hydrophobic interactions and protein unfolding in the binding process.

The purpose of this study was to investigate the interactions of cationic surfactants with oppositely charged gelatin, and to compare the properties of the resulting complexes and the insoluble particles of a gelatin-cationic surfactant, with those obtained with an anionic surfactant, SDS.

Materials

Acid-processed gelatin (type A, bloom 175, having approximate MW, 50 000 (Sigma, USA) and isoelectric point at pH = 8, determined by turbidity measurements), sodium dodecyl sulfate (BDH, England), dodecyltrimethylammonium bromide (Sigma, USA) and cetyltrimethylammonium bromide (Fluka, Chemika, Switzerland) were used without further purification.

Methods

Turbidity measurements

Turbidity measurements were performed by a Ratio/XR turbidimeter, model 43900 (HACH, Ltd). Equal volumes of protein and surfactant solutions, at the same pH, were mixed for 30 s. The turbidity of the resulting solution or dispersion was determined 2 min after mixing.

Binding measurements

The number of bound surfactant molecules per gelatin molecule was determined by using an SDS-specific electrode constructed as described earlier [9, 10]. The concentration of SDS or the cationic surfactants was evaluated from a calibration curves obtained by measuring the voltage of a series of surfactant solutions at known concentrations below the CMC. The voltage reached a constant value, 10 min after the electrode was immersed in the solution (the solutions were stirred by a magnetic stirrer during the measurements). The absolute values of the slopes of the calibration curves were 57–61 mV/decade concentration, and they were not affected by the presence of gelatin at the concentrations used in the present study.

The binding measurements, performed for pre-equilibrated solutions or dispersions (24 h), contained a gelatin at constant concentration (0.3 mM) and various surfactant concentrations. The pre-equilibrated systems were obtained by mixing gelatin and surfactant solutions in a horizontal shaker, at a constant stroke rate and temperature (25 °C) for 24 h. The number of bound molecules was calculated from the difference in surfactant concentration, before and after binding of the surfactant to the gelatin.

Zeta-potential measurements

Zeta-potential measurements were performed with a Malvern-Zetamaster S, model ZEM 5002 (England), using a rectangular quartz capillary cell. The gelatin-surfactant insoluble particles were resuspended in 1 mM NaCl, at the same pH in which they were prepared (24 h after preparation), and the zeta potential was determined at least three times for each type of particles.

The zeta potential was calculated automatically from the measured electrophoretic mobility, by using the Henry equation: $U_e = \varepsilon\, zp\, f/6\pi\eta$, where U is the electrophoretic mobility, ε is the dielectric constant, η is the viscosity and zp is the zeta potential. The Smoluchowski factor, $f = 1.5$ was used for the conversion of mobility into zeta potential [23].

Surface tension measurements

Surface tension measurements were conducted by using a Lauda tensiometer, equipped with a platinum-iridium ring, 10 min after immersing the ring in the measured solution or dispersion, which was prepared 24 h prior to the surface tension measurements.

Hydrophobicity measurements

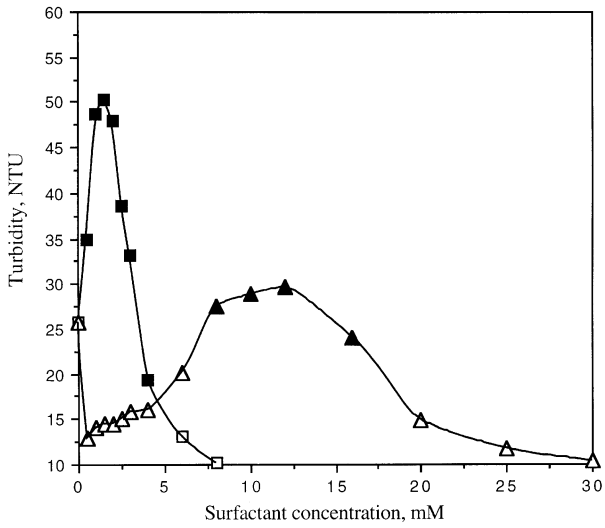
Hydrophobicity of the insoluble gelatin-surfactant complexes was evaluated by measuring the contact angle for the dried complexes by using a Goniometer, model 100–00 230 (Ramé-hart Inc, USA). The samples for the measurement were prepared by mixing equal volumes of surfactant and gelatin solutions, as described for the turbidity measurement. The resulting precipitates were separated from the supernatant (by decantation) and were frozen by liquid nitrogen followed by lyophilization by a Labcono lyophilizer. At surfactant concentration, in which no precipitation occurred, the whole solution was lyophilized. The dry samples were put into a press form (Graseby Specac, Britain) and pressed into a disc by a vacuum press (Perkin-Elmer) at 10 ton during 1 min.

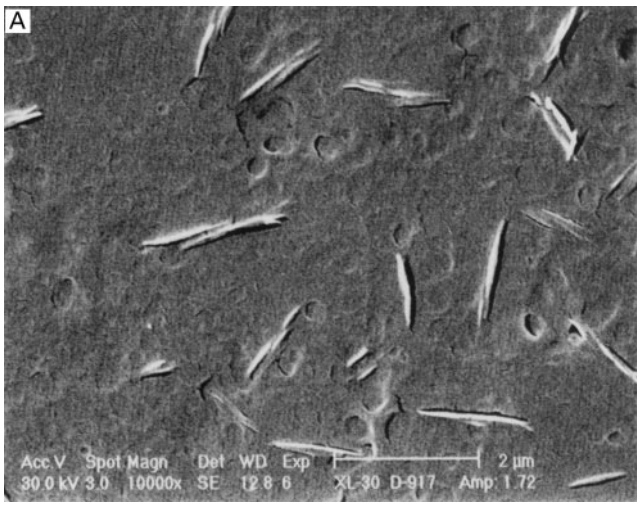
The contact angles of distilled water on the discs, composed of various surfactant-gelatin ratios, were measured at least 4 times for each sample. Since the contact between the water and the discs caused swelling after prolonged periods, all contact angle measurements were performed within 30 s of the time the water droplets were placed on the disc. It is assumed that this measurement would give an apparent contact angle, which could be used for comparison of the various samples.

Results and discussion

The interaction between gelatin and cationic surfactants (DTAB and CTAB) was evaluated by turbidity measurements at pH 9.0, which is above the isoelectric point of the gelatin (pI = 8, determined by turbidity measurements). As presented in Fig. 1, the turbidity, as a function of surfactant concentration, reached a maximum, at 1.5 and 12 mM of CTAB and DTAB, respectively. The maximal turbidity indicates formation of insoluble protein-surfactant particles. By scanning electron microscope, it appears that for both surfactants, at the precipitation range, needle-like colloidal particles were formed (Fig. 2). Such particles were not observed in gelatin or surfactant solutions alone. It should be noted that the colloidal particles were formed at surfactant concentrations at, or above, the CMC (0.5 and 9 mM, for CTAB and DTAB, respectively, as determined by surface tension measurements), contrary to the findings reported previously for SDS-gelatin [9, 17, 24]. This

Fig. 1 Turbidity of gelatin-cationic surfactant systems (in Nephelometric turbidity units, NTU), at various surfactant concentration at pH 9,0. (△) DTAB, (□) CTAB, ▲ and ■ indicate the concentrations in which precipitation occurs after 24 h for DTAB and CTAB, respectively





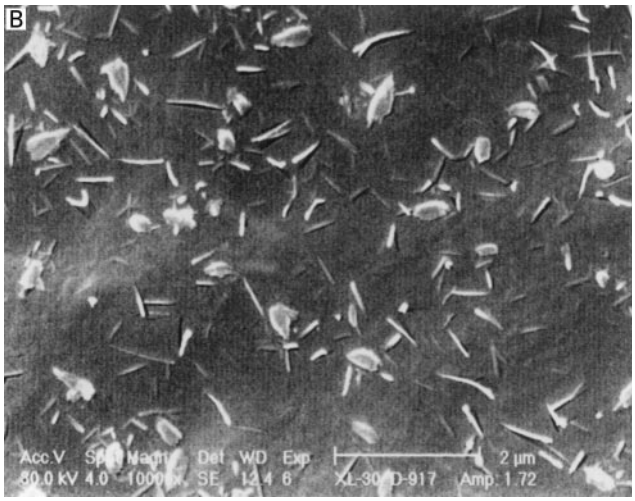


Fig. 2 Scanning electron micrographs of gelatin-surfactant particles, obtained by mixing the gelatin and surfactant solutions at concentration that leads to precipitation. Gelatin concentration 0.75% w/w. (A) at 10 mM DTAB, pH = 9.0, (B) at 1.5 mM CTAB, pH = 9.0

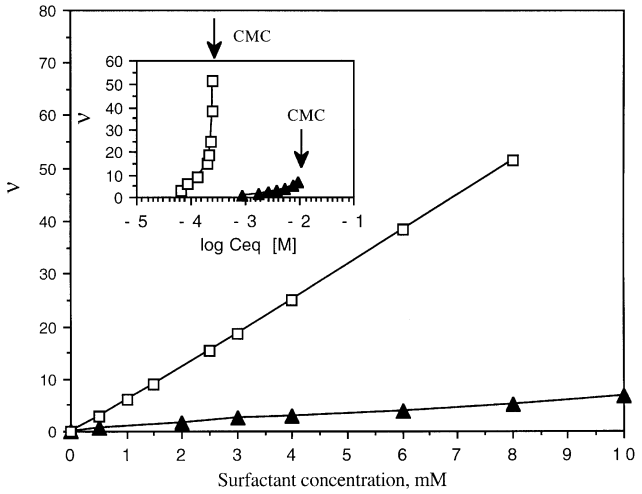


Fig. 3 Binding isotherms (at pH 9.0) of cationic surfactants to gelatin as a function of initial surfactant concentration, and of log C_{eq}. (insert), while C_{eq} is the concentration of free surfactant in solution. (▲) DTAB, (□) CTAB. v is the average number of bound surfactant molecules per gelatin molecule

finding means that at the precipitation range, micelles, and not free molecules interact with the gelatin molecule.

The binding of the surfactants onto the gelatin molecule was evaluated. The binding isotherm (Fig. 3) indicates that at surfactant concentration close to the CMC, a cooperative binding takes place (Fig. 3, insert). This effect is more pronounced for the CTAB.

Since the surfactant and the gelatin are oppositely charged, it may be assumed that the initial interaction between the surfactant and the protein is predominantly ionic, as suggested by several researchers [25–28]. At this stage the surfactant molecules are probably adsorbed via the charged groups, while the hydrophobic tail is extended into the solution. This hydrophobic tail serves as new binding sites for an increased number of additional surfactant molecules. This possibility is demonstrated for binding of SDS to the same type of gelatin, while the protein is either negatively or positively charged (Fig. 4): at pH 4, in which the protein and the surfactant are oppositely charged, after saturation of the ionic binding sites, the cooperative binding begins (close to the CMC). On the other hand, while both the surfactant and the gelatin molecules are negatively charged (at pH = 10) the initial binding is mainly caused by hydrophobic interactions, and the cooperative binding is not promoted.

It is interesting to note that the number of bound surfactant molecules before the cooperative binding takes place is about 5 molecules of DTAB (at pH = 9), as compared to 15–20 molecules of SDS (at pH = 4). This result is in agreement with other reports, which concluded that anionic surfactants are bound more to gelatin than cationic surfactants [21, 22, 29].

The Gibbs energy per bound ligand was calculated from the data for the initial part of the binding isotherms (Fig. 3) by the Scatchard equation [30], $v/C_{eq} = K/n - v$), where v is the number of surfactant molecules bound per protein molecule, C_{eq} is the equilibrium surfactant concentrtion in solution, K is the intrinsic binding constant and h is a cooperativity coefficient. A plot of v/C_{eq} as a function of v has a slope K, from which the binding energy per surfactant molecule, ΔG_v can be calculated by $\Delta G_v = -RT \ln K$. It was found that the Gibbs energy per

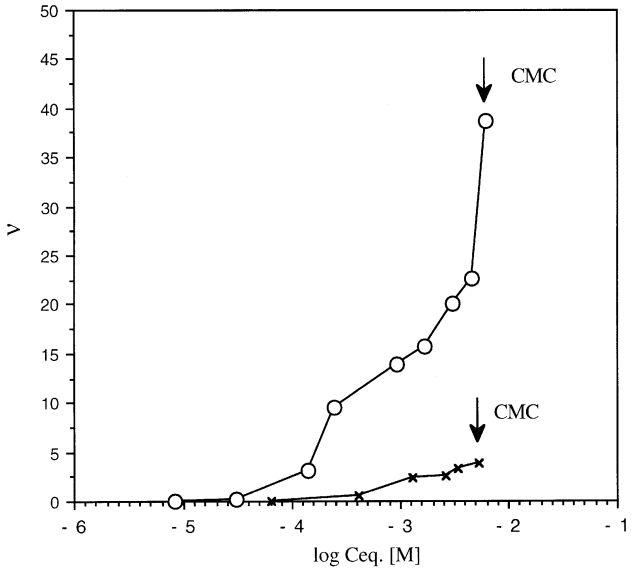


Fig. 4 Binding isotherms of SDS to gelatin as a function of pH. (○) pH = 4.0, (×) pH = 10.0. *v* is the average number of bound surfactant molecules per gelatin molecule

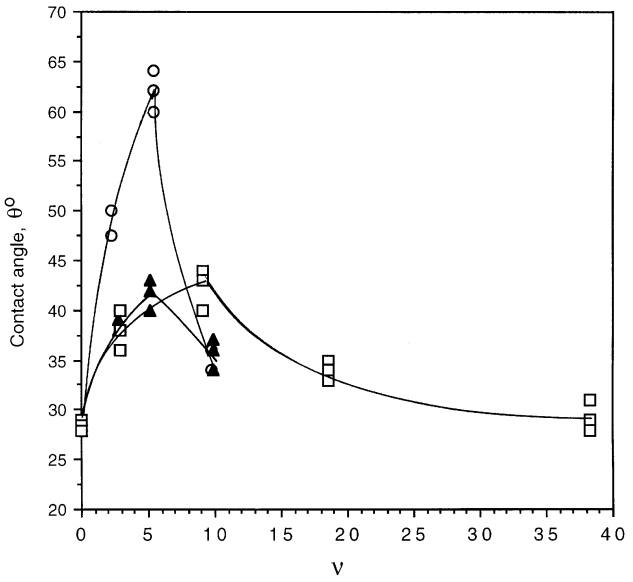


Fig. 5 Contact angle of water on gelatin-surfactant complexes prepared at pH 6.1 (for SDS) and pH 9.0 (for DTAB and CTAB), as a function of the number of bound surfactant molecules per gelatin molecule, *v*. (○) SDS (▲), DTAB, (□) CTAB

cationic surfactant molecule bound to gelatin at pH 9.0 is −9.2 and −16.6 kJ mol for DTAB and CTAB, respectively (correlation coefficient of the linear plots were above 0.97). This result can be explained by the findings [31] that ΔG binding decreases with the increase in the number of bound ligands, since the evaluation of binding energy was limited to $v = 4$ and $v = 15$ for DTAB and CTAB, respectively.

The hydrophobicity of the various gelatin–surfactant systems was evaluated for SDS, CTAB dn DTAB by measuring the apparent contact angle of the dried complex or precipitated particles. As presented in Fig. 5, for all three surfactants, the contact angle reached a maximum value at a narrow range of concentration. The maximal hydrophobicity for gelatin-cationic surfactant (pH = 9) was observed at the same concentration range in which complex precipitation occurred, in accordance with the turbidity data (Fig. 1). The maximal hydrophobicity of SDS-gelatin (pH = 6.1) complex was also observed at concentration range in which precipitation took place [9]. It is interesting to note that the SDS-gelatin particles were much more hydrophobic than the CTAB-gelatin and the DTAB-gelatin particles. This difference in hydrophobicity can be explained by the different binding mechanisms: While the SDS-gelatin particles are formed below the CMC (and before the cooperative binding), the CTAB and the DTAB-gelatin particles are formed close to the CMC (at the cooperative binding region). In the former case, it can be assumed that the charged SDS group is bound to the cationic sites of the gelatin, and the hydrophobic tails are extended into solution, or interact with other hydrophobic chains, which are also bound onto the gelatin molecule. In comparison, for DTAB and CTAB, it is most likely that the surfactant's micelles are bound to the anionic sites of the gelatin (at the precipitation range), while most of the hydrophobic tails are not present on the surface of the particles.

The surface tension of gelation/SDS and gelatin/DTAB complexes were measured, and are presented in Fig. 6, as a function of the number of bound surfactant molecules per gelatin molecule, *v* (*v* was determined prior to the surface tension measurements).

These results show a break in the γ vs. *v* curve, which may indicate the presence of the first CMC, which was also observed by other researchers [15, 24], (below the CMC of the surfactant alone) while plotting γ vs. log surfactant concentration in solution. It is interesting to note that as shown in Fig. 6, CMC 1 occurs for both surfactants at a similar number of bound molecules, $v \approx 2$. This also occurs below the number in which precipitation or maximum hydrophobicity are obtained. Therefore, it can be assumed that the formation of polymer-bound aggregates (at CMC (1)) is dependent mainly on the number of hydrophobic tails, which are bound to the protein molecule. This state may be prerequisite for spontaneous formation of the protein-surfactant insoluble particles.

The zeta potentials of the various surfactant-gelatin particles were measured (at surfactant concentration range which caused precipitation), as shown in Table 1.

In general, it was found that the values of zeta potential were low, as could be expected if partial charge neutralization of the gelatin molecule takes place. However, there were several differences in the various surfactant-gelatin systems: The SDS-gelatin particles were positively charged (pH = 6.1), and there was no charge reversal after binding of SDS. Moreover, the increase in the average number of bound SDS molecules from 2.2 to 7.1, had no effect on the zeta potential. This result could mean that the composition of the surface of the particles is constant, or that the potential is determined mainly by the gelatin molecules. The gelatin molecule (MW 50 000) has about 40 positive charges, and therefore, even at a higher number of bound SDS ($v = 7.1$), less than 10% of the positive charges are neutralized. This result is different from that reported by Chen and Dickenson [18, 19], where the maximal precipitation was obtained at the same concentration in which charge neutralization occurred, while using another surfactant, ethoxylated sodium lauryl ether sulfate and gelatin A. Based on the contact angle measurements, it can be concluded that the SDS-gelatin precipitation resulted from both partial charge neutralization and increased hydrophobicity of the gelatin–SDS complex.

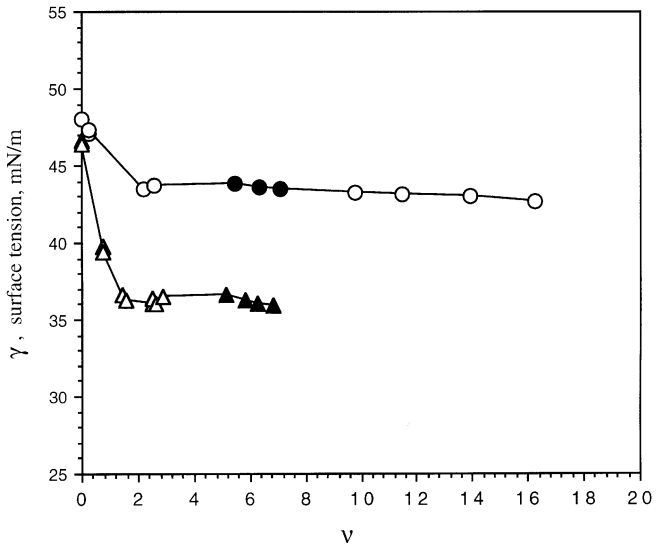


Fig. 6 Surface tension of gelatin-surfactant complexes, as a function of the number of bound surfactant molecules per gelatin molecule, v. (○) SDS (pH = 6.1), (△) DTAB (pH = 9). ● and ▲ indicate the range in which precipitation occurs for SDS and DTAB, respectively

In the DTAB-gelatin particles, there was also no charge reversal (the protein, at pH = 9 is negatively charged). However, the zeta potential decreased from −4 to −0.6 mV, for $v = 5.3$ and 22.7, respectively. Since gelatin has about 59 negative charges, it can be expected that at the highest binding ratio, $v = 22.7$, a significant charge neutralization will occur (since the net charge of the protein affects the zeta potential).

For the CTAB-gelatin particles, a charge reversal was observed, although the maximal binding ($v = 18.5$) was close to that of the DTAB ($v = 22.7$), in which no charge reversal occurred. Since the hydrophobicity of both types of particles is similar, it can be assumed that whatever differences there are, they are caused by the different conformation of the complex and a different composition of the particles' outer layer.

At present, the possibility of using the insoluble particles of gelatin-cationic surfactants as the coating layer in a microencapsulation process, is being evaluated.

Table 1 Zeta potential of precipitated gelatin-surfactant particles, at various numbers of bound surfactant molecules per gelatin molecule

Type of surfactant	pH	C, surfactant concentrtaion, which causses complex precipitation [mM]	v, number of surfactant molecules bound per gelatin molecule	Zeta potential [mV]
SDS	6.1	0.5	2.2	8.2 ± 0.2
		1.0	3.8	8.1 ± 0.3
		1.66	5.4	9.1 ± 0.4
		2.0	7.1	9.3 ± 0.3
DTAB	9.0	8.0	5.3	− 4.0 ± 0.3
		10.0	6.9	− 2.7 ± 0.3
		12.0	9.9	− 1.9 ± 0.2
		16.0	22.7	− 0.6 ± 0.3
CTAB	9.0	0.5	3.1	− 2.7 ± 0.3
		1.5	9.1	0.4 ± 0.2
		3.0	18.5	3.5 ± 0.4

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